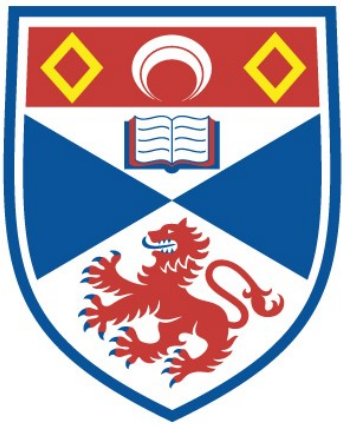
ASPECTS OF PHOTOSYNTHESIS OF AQUATIC MACROPHYTES

Michael Terence Devlin Carr

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1981

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A thesis submitted for the Degree of Doctor of Philosophy (Ph.D.) at the University of St. Andrews by Michael Terence Devlin Carr B.Sc., M.I. Biol., admitted as a Research Student under Ordinance General No. 12 from 1st October, 1972.

ABSTRACT

The measurement of photosynthesis of submerged aquatic macrophytes was investigated with particular reference to water movement over a leaf surface.

Light and dark ^{14}C incorporation rates of two broad-leaved pondweeks, Potamogeton perfoliatus and P. praelongus, were measured in the laboratory using procedures developed for use during in situ field productivity estimates. These measurements are used to evaluate the errors involved in the estimation of photosynthesis rates by this method and to provide recommendations for reducing these errors.

Large variations in the ^{14}C uptake, of replicate leaves or cut discs, were correlated with the position of the leaf on the stem and with the position of the disc on the leaf. Variation due to the size of the disc and to the effect of cutting were much less pronounced.

Measurement of the rate of release of previously incorporated ^{14}C from leaves showed that the release of carbon dioxide is greater in the dark than in the light, suggesting that extensive refixation is occurring and that this method will overestimate net photosynthesis and underestimate gross photosynthesis in light/dark bottle experiments.

The movement of $^{14}CO_2$ from the roots to the leaves of a plant was shown to be small and it is concluded that this may be disregarded as a significant source of carbon dioxide for photosynthesis.

The ratio of leaf area to fluid volume in experimental enclosures was shown to correlate with the size of the pH changes caused by photosynthesis. Changes in pH occurring during typical in situ experiments were shown to be significantly larger than those occurring naturally and it is recommended that large enclosures with small quantities of leaf tissue are used.

Reynolds number calculations shown that laminar boundary layers might be expected to predominate for broad leaves in both the aquatic and terrestrial situation. Theoretical boundary layer thicknesses, for leaves of similar sizes at similar bulk fluid velocities, show that the laminar boundary layer in water will be approximately four times less than that in air.

Turbulent flow produced increases of more than 40% in measured ^{14}C incorporation over unstirred enclosures. Different laminar flow rates over the surface of leaf discs produced measurable changes in the rate of ^{14}C incorporation, showing a correlation between laminar boundary layer thickness and the rate of $^{14}CO_2$ uptake. These measurements show that the diffusion of free carbon dioxide across the average laminar boundary layer would not be fast enough to support the flux of ^{14}C, which must be assisted by the diffusion of the bicarbonate ion.

I Michael Terence Devlin Carr declare that this thesis has been completed by myself, that the work of which it is a record has been done by myself and that it has not been accepted in any previous application for a higher degree.

December, 1981.

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A thesis submitted for the Degree of Doctor of Philosophy (Ph.D) at the University of St Andrews by Michael Terence Devlin Carr B.Sc., M.I.Biol., admitted as a Research Student under Ordinance General No. 12 from 1st October, 1972.

CERTIFICATE

I certify that Michael T.D. Carr has spent 12 terms of research under my direction, that he has fulfilled the conditions of Ordinance General No. 12 and Resolution of the University Court 1967 No. 1, and that he is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

D.H.N. Spence,

St. Andrews, December 1981.

ACKNOWLEDGEMENTS

My gratitude must first go to my supervisor Professor D.H.N. Spence as without his patient guidance, enthusiasm and personal kindness this thesis would never have been completed.

Thanks are also due to the staff of the Botany Department at St Andrews for their assistance while this study was carried out.

CONTENTS

CHAPTER ONE

INTRODUCTION

Aquatic macrophytes include members of the Charophyta, Bryophyta, Pteridophyta and Spermophyta. They play an important role in aquatic eco-systems, providing food, shelter, and a variety of habitats for a large number of organisms. They can be beneficial to man in the maintenance of clean water and the recovery of polluted water. However, in disturbed or newly constructed water bodies rampant growth of aquatic plants may interfere with man's use of freshwaters (Cook, 1974) such that management by removal and ensilage for animal feedingstuffs has been considered (Linn, Staba, Goodrich, Meiske, and Otterby, 1975).

They are mainly hydrophytes, with their perennating buds below the water surface (Spence, 1967), and include the family Potamogetonaceae with the one genus Potamogeton. This genus, the richest in species of all the native aquatic genera (Arber, 1920) has nearly one hundred species, chiefly from freshwaters (Clapham, Tutin and Warburg, 1952). Two species of this pondweed genus, Potamogeton perfoliatus and Potamogeton praelongus wulf, have been considered in the present study. They both have broad submerged leaves that are thin and translucent and grow on bottom muds of fine silts. It is on these fine silts that great forests of the big Potamogetons such as P. perfoliatus, P. praelongus and P. pusillus can be encountered (Macan and Worthington, 1972). The rooted depths of P. praelongus

and P. perfoliatus are in the range 1-3m with the means being 2·2m and 2·06m respectively (Spence, 1964). Both species have been recorded as deep water species by West (1905) ranging from 1·8 to 6m water. Broad leaved Potamogeton species never predominate and rarely occur in water less than 1m deep (Spence, 1967). Plants of P. perfoliatus have been reported as exceeding 4m in length (Spence, 1964) and P. praelongus plants as nearly reaching 8m in length (West, 1905).

Both these two species grow in the limestone lochs of the Durness area. These lochs are classed as calcareous, rich, (Spence, 1967) and have low attenuations of light (Spence, Campbell and Chrystal, 1971) allowing submerged plants to penetrate to 6m (Loch Croispol) or as much as 10m (Loch Borralie) (Spence, 1972). The low phytoplankton populations in these lochs are responsible for this deep penetration of light and encourage the productivity of rooted macrophytes. The farming practices adopted on the small catchment areas of these lochs result in little, if any, run off of soluble fertilizers such as nitrate and phosphate. Addition of nutrients such as these to the water body might be expected to promote phytoplankton population densities and hence restrict light penetration and macrophyte growth.

As the farming practices adopted may not remain constant but can change with different management, it must be of considerable ecological importance to study the production of these macrophytes in this relatively unique situation.

The net production of aquatic macrophytes can be estimated from observed changes in the biomass, providing there are no significant losses from the plants other than respiration (Westlake,1963,1965). The changes in biomass during a specified season, usually a year, can be measured by collection of quadrats using the techniques of aqualung diving. However, the difference between standing crop and biomass is important because of the significance of roots and undergound storage organs (Westlake, 1963). The biomass, rather than standing crop, of aquatic macrophytes can be measured by ensuring collection of all underground parts of the plants in a quadrat (Jupp and Spence, 1974). The dry organic weight, determined from the dry weight and the ash weight, is generally accepted for the determination of biomass for aquatic macrophytes (Wetzel, 1965). The ash weight, although small, is variable among species and environmental conditions (Westlake, 1963) particularly when encrustation is excessive (Wetzel, 1960). This problem, the losses of plant material, and difficulties in ensuring collection of all underground parts of the plants, leads to the agreement that rates of growth, preferably <u>in situ</u> measurements of photosynthetic productivity,are superior to biomass estimates (Wetzel, 1965). They can also be used in the field and the laboratory to predict plant response to sets of environmental variables (Spence and Campbell, 1971).

The standard radio-carbon technique (Steeman-Neilsen, 1952), for measurement of the carbon fixation rate and hence photosynthetic productivity, has been used with

submerged freshwater macrophytes (Wetzel, 1964; Spence and Campbell, 1971). The *in situ* rates obtained by Spence and Campbell (1971), for single detached leaves, expressed as moles x 10^{-7} of carbon per unit leaf area are given in Table 1.1. The range of these results, compared to the range of rates of carbon fixation for terrestrial plants, summarised by Zelitch (1971), shows them to be low. These results and those of Campbell (1972) indicate that productivity is low in water lacking much phytoplankton and having low attenuation coefficients for light.

The broad leaved pondweeds appear to have measured *in situ* rates of carbon fixation outside the range found for terrestrial leaves. A better comparison would be to compare the value for the poorest terrestrial performer, Maple, at a light intensity similar to that found for *P. praelongus* (Campbell, 1972), using data from Hesketh(1963 and Hesketh & Moss(1963) for net photosynthesis of Maple at different light intensities. This gives Maple as $4{\cdot}8 \times 10^{-7}$ moles CO_2 $cm^{-2}h^{-1}$ and *P. praelongus* as $2{\cdot}1 \times 10^{-7}$ moles CO_2 $cm^{-2}h^{-1}$. This is still less than half the poorest terrestrial rate.

However, the underwater light regime suffers from selected attenuation of different wavelengths of light, and in calcareous waters of this clarity the longer wavelengths are absorbed more quickly. Therefore, the underwater light will have, on average, more energy in each quantum and when compared to terrestrial light of the same intensity will have less quanta. The measurements

Table 1.1

A comparison of ^{14}C 'in situ' rates of fixation of broad leaved submerged Potamogeton species (Spence and Campbell,1971; Campbell,1972) with fixation rates for terrestrial plants at high light intensity and 300 ppm CO_2 (Zelitch,1971). All the rates are expressed as net moles x 10^{-7} CO_2 per cm^2 leaf area for single leaves.

	Net fixation
Maize	104 - 143
Sunflower	84 - 99
Wheat	39 - 70
Oak	22.7
Maple	13.6
P. perfoliatus	2.7 - 3.1
P. praelongus	1.55 - 2.1

and calculations for these *in situ* experiments (Spence, Campbell and Chrystal, 1971; Spence, 1972; Spence and Campbell, 1971) give the respective energies per Einstein of quanta as 223 kJ for the loch environment and 200 kJ for the terrestrial. Assuming a relation between the rate of photosynthesis and the number of incident quanta then rates of carbon fixation by aquatic leaves should be about 10% less than equivalent aerial leaves. This difference would not account for the low rates found for *P. perfeliatus* and *P. praelongus*.

The broad leaved pondweeds with thin translucent leaves, have a relatively high specific leaf area (S.L.A.). This is shown in Table 1.2 giving a comparison of net photosynthesis rates, chlorophyll concentrations, and S.L.A. between oak seedlings (Jarvis, 1964) and *P. praelongus* at the same light intensity. The chlorophyll concentrations are similar but both net photosynthesis and specific leaf area are different. The aquatic leaf has a S.L.A. of more than twice the terrestrial leaf which will therefore be approximately twice as thick as the pondweed. The difference may in part be due to the requirement of a terrestrial leaf to support itself. The light path and scattering through the thicker leaf will be increased resulting in absorption of more light. Monteith (1973) suggests that leaves with a chlorophyll concentration of greater than 4 mg dm^{-2} will have an absorption of 80-90% of the incident light and this might be typical of a shade oak leaf. However, *P. praelongus* leaves, with their thinner leaves and slightly lower

Table 1.2

A comparison of net photosynthesis, chlorophyll and specific leaf area (S.L.A.) measurements for Oak seedlings (Jarvis,1964) and *P. praelongus* (Spence and Campbell,1971), at the same light intensity.

	Chlorophyll	Photosynthesis	S.L.A.
	mg dm^{-2}	moles x 10^{-7} CO_2 $cm^{-2}h^{-1}$	cm mg^{-1}
Sun Oak	2.42	11.0	0.21
Shade Oak	4.12	14.7	0.26
P. praelongus	3.24	2.1	0.50

chlorophyll concentration will have a lower absorption of incident light. It is unlikely to be below half that of oak and this assumption would give the following comparison of relative photosynthesis based on absorbed light of 14·2 J $cm^{-2}h^{-1}$ rather than incident: Shade oak 16·3 x 10^{-7} moles C $cm^{-2}h^{-1}$ and P. praelongus 5·3 x 10^{-7} moles C $cm^{-2}h^{-1}$. This still shows the Potamogeton species as having a poor rate of fixation of carbon.

It would, therefore, appear that the low rates of carbon fixation for broad leaved pondweeds (Spence, 1972; Spence and Campbell, 1971; Wassink, 1975; Westlake, 1975; Talling, 1975) are still low when the effects of the underwater light climate and those of leaf morphology are considered. Other aspects of the aquatic environment may be responsible for the low rates, the in situ methods used may give misleading information or it may be that the plants themselves have adapted to low productivity rates.

These short term physiological studies, in the field, are undertaken to measure a plants response to a set of environmental variables. The particular physiological function of carbon dioxide fixation can be used to investigate the productivity of submerged freshwater macrophytes in different lakes.

The aims of this investigation are to examine the techniques used for in situ and laboratory, light and dark, ^{14}C incorporation experiments and to put forth specific recommendations to deal with problems arising from variations in the methods used. To compare the

exogenous carbon environment of natural waters with the terrestrial situation and in particular the differences that arise as a result of enclosure of defined volumes during *in situ* photosynthesis experiments. To examine the movement of fluid over a leaf surface with particular reference to the comparison of laminar and turbulent flow in air and water, and the effect of any boundary layers formed on the supply of exogenous carbon dioxide.

CHAPTER TWO

THE TECHNIQUES OF MEASUREMENT OF PHOTOSYNTHESIS OF AQUATIC MACROPHYTES

2.1 Introduction

Methods of measurement of *in situ* photosynthesis of aquatic macrophytes are limited to certain techniques which can be considered as two main methods of approach.

Firstly, those which monitor some aspect of the environment which is altered in proportion to the amount of photosynthesis occurring in it. For example, in a stream or river, measurement of the dissolved oxygen concentration upstream and downstream of an aquatic weedbed (Westlake, 1967). If the flow rate of the stream can be determined and allowance made for non-photosynthetic organisms, then the rate of oxygen production or consumption of the weed can be estimated. This can be done continuously and net photosynthesis estimated for days, weeks, etc. and these rates can be related to the natural variations in environmental factors such as illumination and temperature. However, using this technique, it is not possible to control or vary the environment easily and experimentation would be limited. In the lake environment this method of using an environmental gradient is not so easy to apply as there is a general lack of unidirectional currents.

The second approach is to enclose parts of, or whole plants, in experimental vessels to define a volume in which a concentration change can be estimated. This has the immediate advantage that the environment may be modified or controlled to suit the experiment and there

is also no dependence on currents. The dissolved oxygen concentration changes can be measured by an oxygen electrode or a Winkler titration. Dissolved carbon dioxide changes can be measured with pCO_2 electrodes and carbon dioxide incorporation into the plant material measured using ^{14}C.

The oxygen measurements are affected, in the case of freshwater angiosperms, by the presence of internal air spaces or lacunae. There can be a lack of correlation between internal and external dissolved oxygen concentrations (Billings and Godfrey, 1967; Wetzel, 1965; Hartman and Brown, 1967) although, recently, Allen and Spence (1981) have shown that this method can be very effective, with rapid equilibration occurring between external and internal oxygen concentrations. The pCO_2 electrode can be complicated by the carbon dioxide/bicarbonate equilibria and also by bicarbonate use by the plant (Steeman Nielsen, 1947; Black, 1972; Raven, 1970; Allen and Spence, 1981; Bowes, Holaday, Van and Haller, 1977). A pCO_2/pH stat method might be employed but this would not be very applicable for use in the field, where ease of replication of experiments is required to provide useful information. A suitable compromise would be enclosure of the plant material and measurement of ^{14}C incorporation.

The measurement of carbon uptake, by the ^{14}C incorporation method used here, was first described by Steeman-Nielsen (1952) for measurement of the productivity

of microscopic algae. This was further developed for use with marine algae (Drew and Larkum, 1967; Johnston and Cook, 1968) and for freshwater angiosperms (Wetzel, 1964; Goldman, 1963). The technique has been used on various parts of freshwater angiosperms, from whole plants to single detached leaves (Campbell and Spence, 1971; Spence and Campbell, 1971; Campbell, 1972; Black, 1972).

The technique was adopted for the present study for two main reasons. Firstly, the use of the technique in the field and the laboratory had been established (Spence and Campbell, 1971). Secondly, it has an inherent high level of sensitivity (Goldman, 1968). ^{14}C labelled bicarbonate can be supplied by the Radiochemical Centre with a specific activity of 60 mCi/mmol, which gives $2{\cdot}22 \times 10^{9}$ cpm/mmol of bicarbonate. Using the Tracerlab gas-flow counter a count rate of 50 cpm above background would be a reasonable minimum below which counting accuracy becomes more difficult to maintain. If we assume a counting efficiency of only fifty per cent then each sample would have to produce 100 cpm above background. This count would be produced by the presence of $2{\cdot}22 \times 10^{-10}$ moles of bicarbonate, with a specific activity of 60 mCi/mmol, in a sample. This is approximately 10^{-4} of the rates of photosynthesis, expressed per cm^2 leaf area per hour, of aquatic angiosperms.

2.2 The Plant Material

Fresh shoots of *P. praelongus* and *P. perfoliatus* from the Lake of Menteith and from Loch Drumore, were collected from the loch bottom by aqualung diving. Individual shoots were picked by hand and put in a nylon net 'shopping' bag. Repeated dives were made from the boat and full nylon bags stored in shaded plastic buckets containing loch water, in the boat. These were transferred to a Land Rover and taken to the laboratory.

Whole plants, including roots, from these lochs and from Loch Croispol were planted in 7 cm of garden soil in plastic bins. The soil was covered with a layer of sand and the bins filled with tap water. These bins were kept in the greenhouse, which was shaded in summer and had supplementary illumination, provided by mercury vapour lamps, during the winter. By changing the water and scraping the sides of the bins, algal growth was kept to a minimum.

Whole shoots, detached whole leaves, or discs cut from leaves, from these two sources of materials were used for individual experiments. However, the choice of individual leaves used in any experiment might have a significant effect on the resultant incorporation of ^{14}C and the following series of experiments were performed to provide a guide to the selection of plant material.

The effect of the position of a leaf on a shoot and the position of a disc cut from the leaf, on the measured incorporation of ^{14}C in the light by P. perfoliatus.

Experimental Shoots of P.perfoliatus were collected, in black plastic bags containing lake water, by aqualung diving from the Lake of Menteith, and taken directly to St. Andrews. They were placed in buckets of fresh lake water, in a shaded greenhouse, until required for the experiment the following day. At no time during this operation were the shoots exposed to direct sunlight.

A healthy shoot was washed in the incubating medium of 2×10^{-3}M $KHCO_3$ and 1×10^{-5}M $CaCl_2$, which had been freshly made up from deoxygenated distilled water and stored at the temperature (15°C) of the experiment. Using an elastic band, a brass weight was attached to the base of the shoot and it was placed in a 1·8 l Kilner jar filled with incubating medium, and sealed to exclude any air. FiftyμCi of $NaH^{14}CO_3$ were injected through the rubber seal in the lid and the jar was shaken quickly to thoroughly mix the isotope before placing it upside down in the water bath as in Figure 2.1. The 150 watt lamp above the jar was switched on for the beginning of the two hour incubation period.

At the end of the incubation, the lamp was switched off, the shoot removed and washed thoroughly in distilled water several times. Starting at the base of the shoot the leaves were removed and using the cork borer discs of 6 mm diameter were cut out. These discs were stuck onto

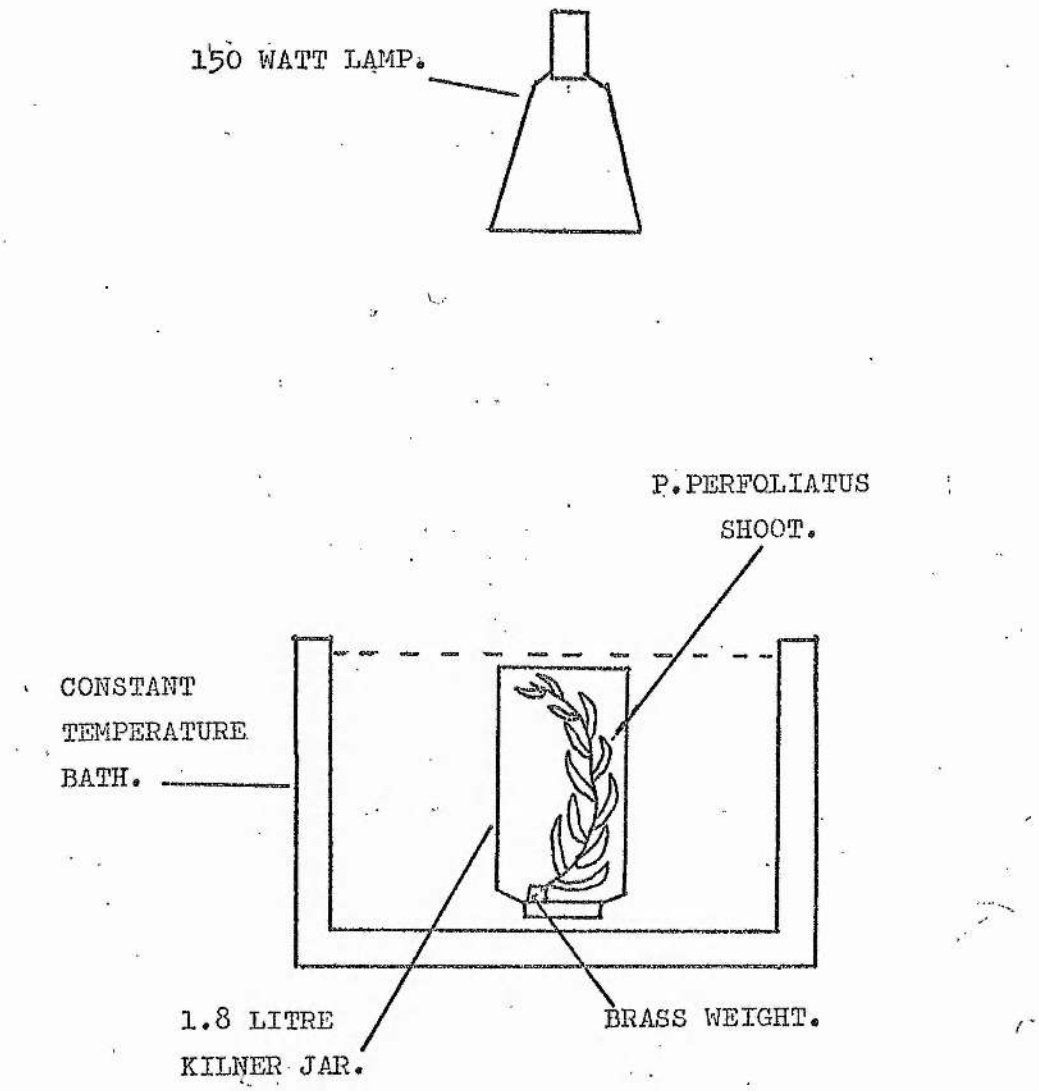


Figure 2.1

The experimental apparatus used to investigate the effect of the position of a leaf on a shoot and the position of a disc, cut from a leaf, on the measured incorporation of ^{14}C in the light by P. perfoliatus.

separate planchettes with a small drop of glycerine albumen, placed on a slide warmer to heat-kill them and the discs covered with acetic acid.

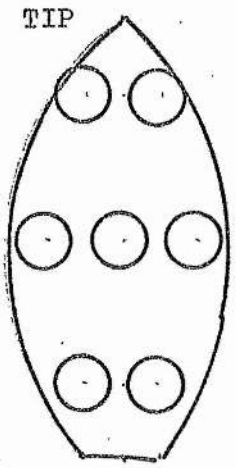
The discs were cut from the leaves in three patterns according to the leaf size (Figure 2.2). Numbering from the base of the shoot leaves 1-9 followed pattern A, 10-11 pattern B and 12-14 pattern C. After the planchettes were dried, the ^{14}C activity in each disc was counted.

Results The cpm-bg for the discs cut from each leaf were averaged and plotted against the relative position of that leaf, on the stem, as in Figure 2.3. This shows that the incorporation of ^{14}C in the light, as measured under these conditions, and expressed per unit leaf area, decreases from the base of the shoot to the tip, when the last fourteen leaves are looked at. Leaves close to the base of this shoot have incorporated nearly twice as much ^{14}C as those at the apex.

The distribution of incorporated ^{14}C within a leaf was considered by comparing the pair of discs cut from the base of the leaf to the pair of discs cut from the apex of the leaf. Leaves 1 to 11 were considered for this as the disc cutting patterns used (Figure 2.2 pattern A & B) include two discs at the base and two discs at the apex of the leaf. The average cpm-bg for the basal pair of discs are given for each leaf in Figure 2.4. This shows that the apical pair of discs incorporated more ^{14}C than the basal pair, and there is a tendency for the basal pair to decline more quickly the closer the leaf is

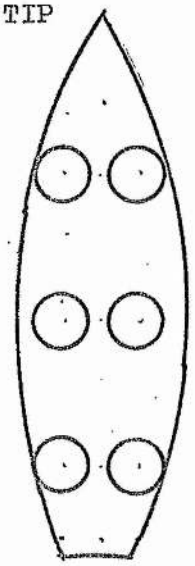
A

THE POSITION OF DISCS CUT FROM LEAVES 1 TO 9. (numbered from the base)



B

THE POSITION OF DISCS CUT FROM LEAVES 10 AND 11.



C

THE POSITION OF DISCS CUT FROM LEAVES 12 TO 14.

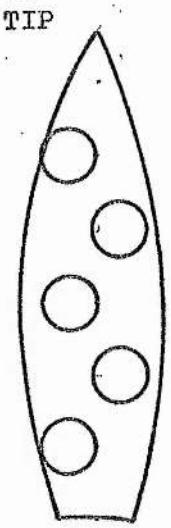


Figure 2.2

The three patterns of discs cut from leaves of P. perfoliatus used to detect an effect of position of disc on the measured ^{14}C incorporation in the light.

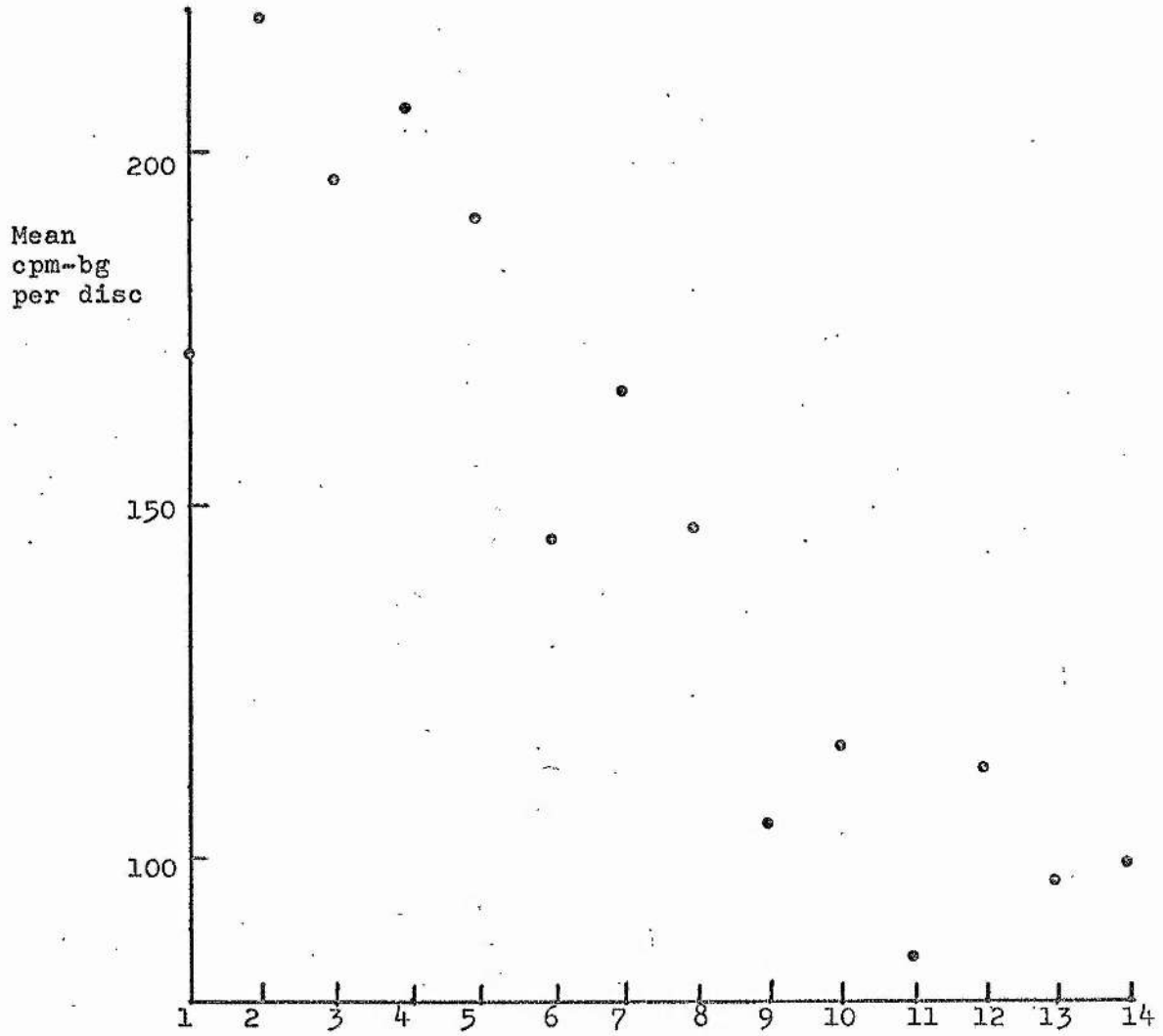


Figure 2.3

The effect of the position of a leaf on a shoot of *P. perfoliatus* on the ^{14}C incorporation in the light. The average cpm-bg for the discs from each leaf are plotted against the position of the leaf on the stem.

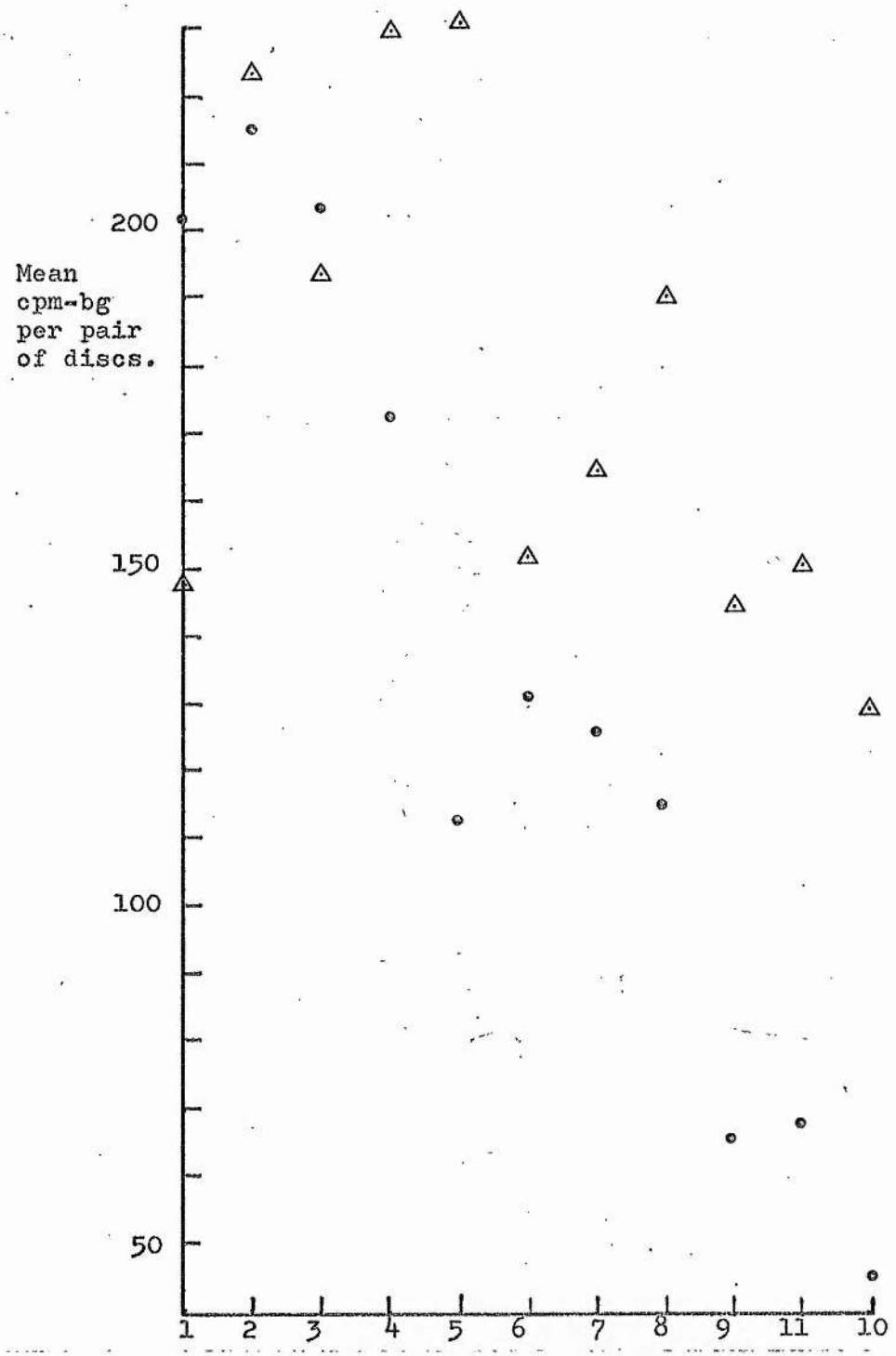


Figure 2.4

The difference in ^{14}C incorporation in the light between discs cut from the base and the apex of a leaf, in relation to the position of the leaf on the shoot. The average cpm-bg for the pair of discs cut from the base is given by ∘ and the apical pair by △ .

to the apex of the shoot.

Discussion Leaves nearer the apex of the shoot are progressively more perfoliate and consequently, a greater proportion of the basal part of the leaf is wrapped around the stem. Self shading of this part would account for the difference in ^{14}C incorporation between the base and apex of a leaf.

Visual observation of the leaves showed the base of each leaf to be more transparent than the apex and also leaves nearer the tip of the shoot were more transparent than those at the base. It is reasonable to assume that increasing transparency of a leaf will be caused by a higher specific leaf area and a lower chlorophyll content and that this explains the observed trends in ^{14}C incorporation of leaves along a shoot.

Thus, the morphology of a particular leaf may be more significant in determining the ^{14}C incorporation than its position on the shoot relative to the incident light.

The effect of using discs of different sizes, from leaves of P. praelongus cut before and after the period of incubation, on the measured incorporation of ^{14}C in the light.

Experimental Leaves were excised immediately prior to requirement for each part of the experiment, from P. praelongus plants grown in a single artificial pond in the greenhouse. Thus, all the leaves had the same

light, temperature and water chemistry pre-treatment. The experimental enclosure was a perspex box, with an outlet at one end and an inlet at the other. The end plate was removable and was held with a series of bolts, using a rubber gasket to seal it against the end of the box. This allowed access to the tray holding the plant material as in Figure 2.5. The incubating medium was pumped around a system, with a heat exchanger, to maintain the temperature at $15^{o}C$.

The leaves were first washed in the incubating medium of $2 \times 10^{-3}M$ $KHCO_3$ with $1 \times 10^{-5}M$ $CaCl_2$ and ten discs of the chosen size were cut out with a cork borer. Each of these was then attached to a pair of hooked needles which were fixed in a set pattern on the tray (Figure 2.5). This was then quickly inserted into the box, the end plate bolted on and the system pumped full of incubating medium. The air bubbles were then removed from the system and the flow started. The total volume of incubating medium was 1·5 l, one hundred μCi of $NaH^{14}CO_3$ were injected through a 'suba' seal in the system, and the overhead light system was switched on. At the end of the incubation period of one hour, the fluid was pumped out, the end plate removed and the tray withdrawn. The leaf discs were removed and processed for counting in the usual manner. Samples of the incubating medium were taken at the beginning and at the end of the experiment, to determine the specific activity of the carbon.

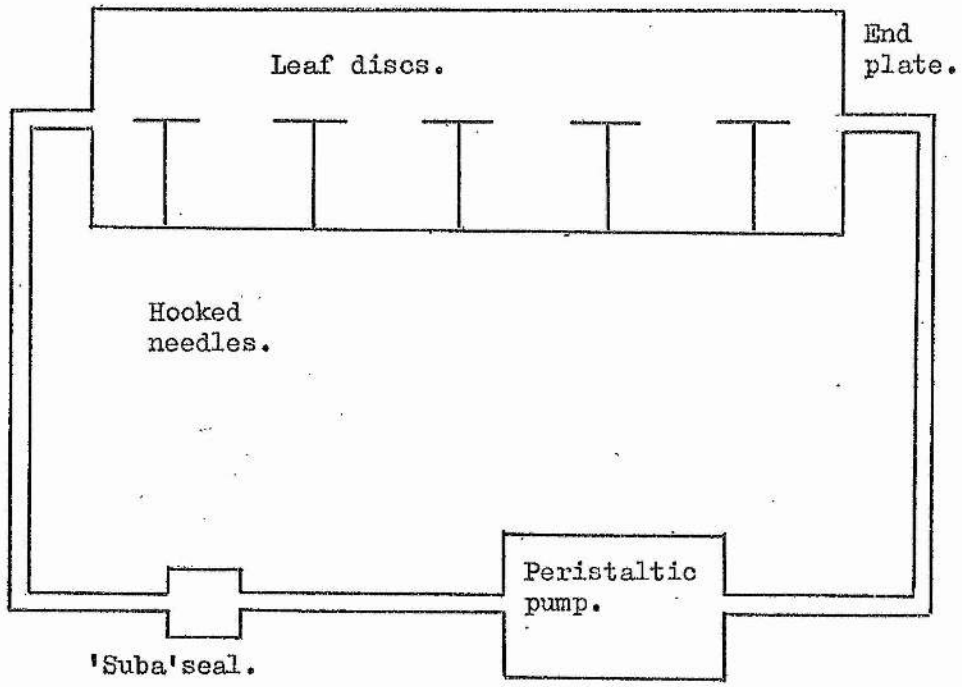


Figure 2.5

The experimental enclosure used to measure the effect of using discs of different sizes, from leaves of P. perfoliatus, cut before and after the period of incubation, on the measured rate of ^{14}C incorporation in the light. Ten replicate leaf discs were aranged in two rows of five.

This procedure was repeated with three sizes of leaf disc of areas 1·0, 2·0, 3·5 cm^2 and finally with whole leaves. Discs of the three sizes were cut out of the whole leaves after the incubation and stuck onto planchettes. The leaf discs and precipitates were weighed and the radioactive counts were corrected for self-absorption.

Results Using the counts obtained from barium carbonate precipitates of known volumes of incubating medium, the number of moles of carbon incorporated per mg leaf dry wt. or per cm^2 leaf area were calculated. These are given for each disc size, cut before and after the incubation in Table 2.6. The standard error of the mean of each set of 10 discs is given.

When discs are cut before incubation the largest (3·5 cm^2) and smallest (1·0 cm^2) sizes gave very similar ^{14}C incorporation rates but the middle size (2·0 cm^2) gave a lower rate. Expression of the results on a unit leaf area basis did not significantly alter this result. When discs were cut out after the incubation the three sizes of disc showed less differences. The cutting of discs before incubation produced low ^{14}C incorporation rates when compared to discs cut after incubation although this effect was less in the largest disc size (3·5 cm^2).

The disc size may have influenced the choice of position or cutting of a disc from a leaf and introduced variability caused by changes in specific leaf

Table 2.6

The average carbon uptake in the light for different sizes of discs of Potamogeton perfoliatus, cut before and after the period of uptake. This is expressed as moles x 10^{-7} CO2/mg dry weight and the range is given as the standard error of the mean. The figures in parentheses refer to the carbon uptake expressed as moles x 10^{-7} CO2/cm^2 disc area.

	Size of disc in cm^2.		
	1.0	2.0	3.5
Disc cut before incubation.	2.32±0.20 (3.55)	1.71±0.13 (2.53)	2.33±0.14 (3.14)
Disc cut after incubation.	3.06±0.48 (4.22)	2.91±0.09 (3.74)	2.47±0.28 (3.96)

area. The size of disc cut before or after incubation appears to have less effect on the incorporation of ^{14}C than the choice of the plant material itself.

The Distribution of measured ^{14}C incorporation in the light, along the axis of a detached leaf.

Experimental The positions of the discs, on the leaf, cut in a previous experiment were noted and a null hypothesis, that the incorporation of ^{14}C measured in a single disc should be equal to the combined incorporation of ^{14}C of the discs of that leaf divided by the number of discs was statistically analyzed.

Results The results of a Chi-squared test of the null hypothesis are shown in Table 2.7. This shows that the null hypothesis fails four times out of six, indicating that a set of discs cut out of the same leaf cannot be used as reliable replicates because there may be variability in ^{14}C incorporation caused by the original position of the disc on the leaf.

Conclusions to plant material investigations

The preceding investigations reveal that the choice of plant material is of prime importance in ^{14}C incorporation experiments in the light. For replication in experiments when it is necessary to use many leaves or discs cut from them, factors such as S.L.A. as well as age, and environmental pre-treatment will have to be considered. Leaves will have to be taken from a similar position on many shoots to reduce the observed variation shown in Figure 2.3. The requirement for many replicate

Table 2.7

The results of the Chi squared analysis of the distribution of ^{14}C in discs, incorporated in the light, along the axis of leaves.

	Leaf number.					
	A	B	C	D	E	F
No of discs cut.	4	4	5	5	4	4
Chi squared.	1.4	0.53	0.11	2.09	0.04	0.68
Degrees of freedom.	3	3	4	4	3	3
Probability.	0.7	0.9	0.99	0.65	0.99	0.85
Null hypothesis.	false	false	true	false	true	false

tissue samples is not solved by taking discs from leaves as the position of cutting can significantly affect the measured ^{14}C incorporation, Figures 2.4 and 2.7. The pre-treatment and other aspects of the origin of the plant material will be discussed in individual experiments.

2.3 Experimental enclosures

The requirement for the enclosures was primarily that given previously, namely to define a volume of fluid in which the concentration change was to be measured. Although when measuring the incorporation of ^{14}C into plant material it serves to contain and define the concentration of ^{14}C presented to the plant.

Various experimental enclosures were used ranging in volume from 30 ml MaCartney bottles to 2 litre or larger Kilner jar systems. The enclosures which allowed flow of incubating medium over the plant material were all of the recirculating type. The glass or acrylic nature of the enclosures allowed light to reach the plant material inside. To obtain a dark enclosure they were wrapped in aluminium foil or black polythene.

All the experimental enclosures were designed to prevent exchange of $^{14}CO_2$ with the atmosphere and, when filled, care was taken to eliminate all air bubbles from the system, thus preventing any subsequent exchange with the gaseous phase. The exchange of $^{14}CO_2$ with the atmospheric CO_2 was observed to occur at a reasonably fast rate, in the following investigation.

To demonstrate the change with time, in the measured ^{14}C, content of a ^{14}C-labelled bicarbonate solution exposed to the atmosphere.

Experimental To perform this experiment a volume of 2×10^{-2} M $KH^{14}CO_3$ solution was sealed in a MaCartney

bottle. A small amount of isotope was injected and thoroughly mixed by vigorously shaking. Then the top was removed and left off for the whole period, while samples were withdrawn for the precipitation experiment. This procedure took nearly 20 minutes and the order in which the planchettes were used was recorded.

Results The cpm minus background for each planchette is plotted against the sequence of precipitation in Figure 2.8 for one of the series. This shows a gradual decrease in measured activity over the period of only 20 minutes. An identical trend was observed with the two other series of planchettes involved in the experiment.

Therefore, all enclosures were sealed to prevent loss or exchange of $^{14}CO_2$ and all operations involving opening of an enclosure were of short duration.

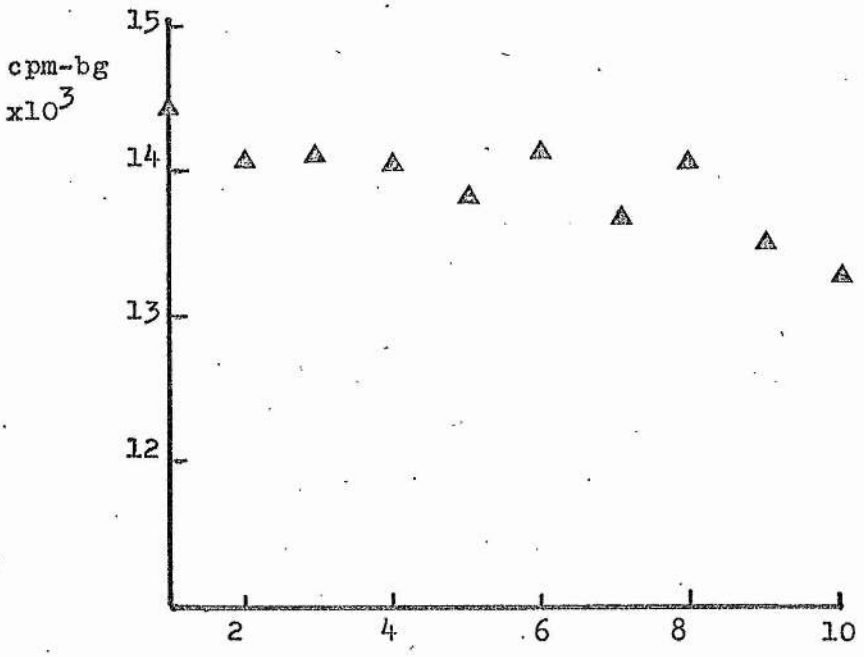


Figure 2.8

The cpm-bg of a sequence of samples withdrawn from a ^{14}C bicarbonate solution exposed to the atmosphere for approximately twenty minutes.

2.4 Isotope Injection

The isotope used in all experiments was $Na^{14}CO_3$ of specific activity 40 mCi/mmol, from the Radiochemical Centre at Amersham. This was supplied in sealed ampoules of 1,000 μCi and the contents were made up to 10 ml total volume with distilled water, to give a stock solution of 100 μCi per ml. These solutions were kept sealed in 28 ml MaCartney bottles which had two holes in the metal cap allowing access to the rubber seal. Working solutions were made up by further dilution to approximately 10 μCi per ml. All pipetting operations using isotopes were done with syringes, through the rubber cap seales of the bottles.

The chosen amount of isotope was added to the experimental enclosure by injection through a seal, into the system. In the case of the bottles this was usually done through the rubber seal in the cap, but in the recirculating systems a 'suba' seal in the circuit was used. Immediately after injection the enclosure was either vigorously shaken or in the case of the recirculating system it was operated for a period required to mix the solution. This period was previously determined for any one system by using coloured dyes and was usually of the order of one minute.

2.5 Precipitation of Carbon from Incubating Media

After the mixing period was complete a small aliquot of the incubating fluid was withdrawn in a syringe. This process was also repeated just prior to finishing the experiment. The carbon in solution in these aliquots was precipitated as the carbonate in a homogenous solid phase to enable counting for ^{14}C.

The initial method of precipitation adopted (Black, 1972) was to add the aliquot of incubating media to an equal volume of saturated barium hydroxide solution directly on to the surface of a planchette. However, the area occupied by the resulting precipitate was variable and non-homogenous. Various methods of containing the precipitate on the planchette were investigated.

The effect of different methods of containing the carbonate precipitate, formed on the planchette, on the resulting radioactive count.

Experimental Samples were taken from a ^{14}C-labelled solution of $2 \times 10^{-4}M$ $KHCO_3$ and precipitated on the surface of aluminium planchettes with an equal volume of saturated barium hydroxide. Three methods of containment of the precipitate were used; glass fibre and lens tissue discs, both of 3·46 cm^2 area, and without either. The samples were precipitated in a rotating sequence between the three methods to remove any bias with regard to time of withdrawal from the main sample. After drying, the planchettes were counted to determine the measurable

radioactivity.

Results The average cpm-bg for each of the three methods of containment is given in Table 2.9. The average area of the precipitates obtained without either lens or fibre tissue is given alongside the area of the discs used.

Each series of planchettes should contain the same amount of ^{14}C but each series shows a different measured count. The lens tissue gives the highest count with the glass fibre lower but not significantly different. The slight difference may be due to increased absorption by the thicker glass fibre tissue. However, in either case the paper weight per unit will have to be used in correcting for self absorption. When no tissue is used to contain the precipitate a significantly lower count is measured. This must be partly due to the fact that the area occupied by these precipitates is less and increased self-absorption gives a lower count. The precipitate in these cases was noticeably non-homogeous, an effect which would increase self absorption relative to its mass per unit area.

The use of lens tissue or glass fibre to define the area occupied by the precipitate is beneficial, as it causes greater homogeneity and reduces the self-absorption by causing the precipitate to occupy a greater area.

This method of precipitation was further tested with solutions containing different molarities of bicarbonate.

Table 2.9

The effect of different methods of containing the carbonate precipitate formed on a planchette, on the resultant radioactive count. The average cpm-bg for ten replicates of each method along with the standard error and the area occupied by each precipitate is given.

	Method of containing precipitate.		
	Lens tissue	Glass fibre	No tissue
Average cpm-bg.	20,055	19,434	13,823
Standard error.	1,184	656	684
Area cm^2	3.46	3.46	1.77

The effect of the molarity of the bicarbonate incubating solution on the radioactive count measured by direct precipitation with $Ba(OH)_2$ onto the planchette

Experimental Four solutions of $KHCO_3$, in concentration from 2×10^{-1} M to 2×10^{-4} M were made radioactive by addition of equal amounts of activity. Then equal volumes of these were precipitated with equal volumes of saturated barium hydroxide, directly onto planchettes, using glass fibre discs to contain the precipitate. This process was repeated to provide replicate samples of each solution. These were then dried and counted. The weights of the planchettes before and after the addition of the precipitate were measured and the counts corrected for self absorption.

Results The average cpm-bg for the replicates of each molarity of $KHCO_3$, along with their standard errors, are given in Table 2.10. These are further expressed as a percentage of the highest count (ie. 2×10^{-4} M $KHCO_3$). There is a distinct correlation between decreasing molarity of the bicarbonate solution and the increasing radioactivity measured.

Discussion Each of the four bicarbonate solutions contained the same amount of ^{14}C per unit volume and as the same amount of each was precipitated the observed counts should be the same. As the counts are corrected for self absorption the increase in counts with decreasing molarity of bicarbonate should not be caused by better counting geometry and less self absorption.

Table 2.10

The effect of the molarity of the bicarbonate incubating solution on the radioactive count as measured by direct precipitation with barium hydroxide onto the planchette. The average cpm-bg for replicate planchettes of each molarity are given with their standard errors. These are further expressed as a percentage of the lowest molarity.

	Molarity of bicarbonate			
	$2x10^{-1}$	$2x10^{-2}$	$2x10^{-3}$	$2x10^{-4}$
Average cpm-bg	2,692	7,737	17,153	18,465
Standard error	558	654	2,992	1,191
Percentage of $2x10^{-4}$	14.6	41.9	92.9	100

The difference between the actual amount of ^{14}C added to each planchette and the measured amount indicates that this method of precipitation is not quantitative. The ^{14}C must be being lost to the gas phase when the precipitate dries out. The success of precipitation of the total inorganic carbon from a solution by addition of barium hydroxide depends upon the solubility product for barium carbonate (7×10^{-9} at 16°C, Handbook of Physics and Chemistry 1974). Thus, although the solubility of barium carbonate will be $\sqrt{7 \times 10^{-9}}$ i.e. $8{\cdot}37 \times 10^{-5}$ mol l^{-1}, addition of excess barium ions must satisfy the following identity:

$$\left[Ba^{++}\right] \times \left[CO_3^{--}\right] \leq 7 \times 10^{-9} \quad \ldots\ldots\ldots\ldots \quad 1$$

The solubility product of barium hydroxide however is 5×10^{-3} (Handbook of Physics and Chemistry 1974) and its solubility will therefore be $\sqrt[3]{\frac{5 \times 10^{-3}}{2}}$ i.e. $1{\cdot}3 \times 10^{-1}$ mol l^{-1}. Thus, addition of an equal volume of saturated barium hydroxide to an incubating solution will introduce a concentration of $6{\cdot}5 \times 10^{-2}$ mol l^{-1} of barium ions. Thus, the maximum concentration of carbonate ions permissible from equation 1, will be $\frac{7 \times 10^{-9}}{6{\cdot}5 \times 10^{-2}}$ which equals $1{\cdot}08 \times 10^{-7}$ mol l^{-1}. As this is about 0·1% of the total carbon present in the least concentrated bicarbonate solution used (2×10^{-4}M $KHCO_3$), in Table 2.10 the precipitation would be expected to be quantitative.

However, this method will be limited by the availability of excess barium ions. For the most concentrated bicarbonate (2×10^{-1}M $KHCO_3$) solution used in Table 2.10 there will be 20 carbonate ions to every 6·5 barium ions and the quantitative precipitation is not possible. It was, therefore, decided to test another more soluble barium salt for its ability to precipitate carbonate from solution.

The efficiency of the barium salts, $BaCl_2$ and $Ba(OH)_2$ as precipitating agents for inorganic carbon from solution

Experimental Fresh saturated solutions of $BaCl_2$ and $Ba(OH)_2$ were used to precipitate equal volumes of a previously made up ^{14}C-labelled bicarbonate solution. This was done directly onto planchettes using glass fibre discs to contain the precipitate. The experiment was repeated using a different ^{14}C-labelled solution and the precipitation was performed in test tubes, with $BaCl_2$ and $Ba(OH)_2$, and directly onto a planchette, with $Ba(OH)_2$. An aliquot of the resuspended precipitate was taken from each tube and put on a glass fibre disc on a planchette. All the planchettes were dried and counted.

Results The cpm-bg for the planchettes from the first part of the experiment are given in Table 2.11a. This shows that, compared to the amount for $Ba(OH)_2$ precipitates the $BaCl_2$ is less than 2% as effective. The second part of the experiment confirmed that (Table 2.11b) and showed that performing the precipitate first in a test tube had little effect on the discrepancy. It further

Table 2.11

a/ The efficiency of the hydroxide and the chloride of barium on direct precipitation, on a planchette, of carbonate from solution.

b/ The efficiency of the hydroxide and the chloride of barium on precipitation in a test tube of carbonate from solution.

c/ The difference between direct precipitation on a planchette and precipitation in a test tube on the efficiency of precipitation of carbonate from solution by barium hydroxide.

a/

	Precipitation agent.	
	$BaCl_2$	$Ba(OH)_2$
Average cpm-bg.	194	11,564
Standard error.	67	794

b/

	Precipitation agent.	
	$BaCl_2$	$Ba(OH)_2$
Average cpm-bg	1,187	23,719
Standard error.	139	3,600

c/

	Method of precipitation.	
	Test tube	Direct
Average cpm-bg	23,719	37,785
Standard error.	3,600	2,841

shows that (Table 2.11c) direct precipitation with $Ba(OH)_2$ was more efficient than precipitation with $Ba(OH)_2$ in a test tube first. This is probably caused by problems introduced by taking an aliquot.

Discussion Therefore, the problem is not solved by using a more soluble barium salt but exacerbated. The explanation for this comes from a consideration of the equilibria of the forms of the inorganic carbon present. In the natural waters and incubating media used only a small proportion will be present in the form of carbonate. Removal of this carbonate by precipitation from the equilibrium will cause interconversion and formation of more carbonate from bicarbonate and carbon dioxide i.e.

$$(OH^-) + HCO_3^- \rightleftharpoons CO_3^{2-} + H^+ (H_2O)$$

This consumption of carbonate causes a reduction in the pH of the solution and the equilibrium position will be shifted away from carbonate to bicarbonate and carbon dioxide. Therefore, an addition of barium ions causes a small amount of carbonate to be precipitated until the remaining inorganic carbon is bicarbonate which is soluble in the presence of excess barium ions. Drying of this precipitate on a planchette will then cause the carbon dioxide to evaporate with consequent loss of $^{14}CO_2$. Thus, the purpose of the precipitation is not satisfied as it is not quantitative (Table 2.10).

However, this analysis explains why the hydroxide rather than the chloride of barium is more effective in

precipitating the carbonate. Addition of the hydroxide increases the pH of the resultant mixture pushing the equilibrium towards the carbonate which can be precipitated before the solution is dried out. The concentration of barium itself can then become limiting however.

To solve these problems a new precipitation technique was adopted for quantitative precipitation. An equal volume of molar sodium hydroxide was first added to the sample, to raise the pH above 10, thus ensuring the carbon present was in the form of carbonate. Addition then of the same volume of saturated barium chloride provided the excess of barium ions which precipitated all the carbonate present. This procedure would have to be carried out in a test tube not directly on to a planchette because of the volumes involved and the effect of the sodium hydroxide upon the amphoteric coating of the aluminium planchettes used. The following experiment was carried out to investigate the methods of putting the precipitate, formed in a test tube onto a planchette.

The efficiency of precipitate collection by vacuum filtering and centrifugation

Experimental Twenty centrifuge tubes were taken and 1 ml of 1M NaOH was added to each. One ml aliquots of ^{14}C bicarbonate were added to each, followed by 1 ml saturated barium chloride. Thus, each centrifuge tube will have exactly the same contents. Every other tube was taken and centrifuged on a bench centrifuge until the

precipitate had collected at the bottom of the tube. The supernatant was decanted and the precipitate was washed in distilled water. This process was repeated. Then the precipitate was resuspended in only 1 ml of distilled water and 0·5 ml of this was put on a planchette, contained by a glass-fibre disc, and dried.

The even numbered tubes were filtered through glass-fibre discs under vacuum in a buchner funnel. The tubes were rinsed out with distilled water and this was used to wash the precipitate. The filter paper was then removed and placed on a planchette and dried.

Results The average cpm-bg for the two series of planchettes are given in Table 2.12. As the centrifuged samples had aliquots of 0·5 ml taken from them, their average count shold be 50% of that of the filtered samples, but as the filtered samples occupy a smaller area, because of the shape of the buchner funnel, they have a greater self-absorption, thus reducing the observed count.

However, the variance of the centrifuged samples, as indicated by the standard error, compared to that of the filtered samples is much greater. This is probably because the centrifuged precipitate contains a certain amount of water and the volume of resuspension then becomes uncertain. When the precipitate was dried out first to remove this, it became impossible to resuspend them properly. The variance of the filtered samples is very low, indicating a good efficiency of collection.

Table 2.12

The efficiency of precipitate collection by vacuum filtration versus centrifugation.

	Method of collection of precipitate.	
	Centrifuged	Filtered
Average cpm-bg	82,182	35,770
Standard error	2,102	100

It was possible to cause the precipitate to be forced through the paper if the vacuum was too high, but if the system was set up carefully this did not happen.

The stability of the precipitates with time was investigated as the barium carbonate might exchange $^{14}CO_2$ with $^{12}CO_2$ in the atmosphere or if the precipitate was hygroscopic, self-absorption would increase due to the presence of absorbed water.

The observed change of measured activity of precipitates with time

Experimental A set of planchettes, the counts of which are given in Table 2.9, were recounted at intervals over a period of several days. During this time planchettes were left exposed to the atmosphere.

Results The change of the average cpm-bg, for the three sets of planchettes is shown in Figure 2.13. There is a small linear decrease of counts with time but after 2·5 days the counts are still over 99·5% of the initial count. Even after 14 days the counts have only dropped to just over 95·6%. As all the planchettes are counted within hours, after precipitation, rather than days, it would be reasonable to ignore any loss of counts from exchange with the atmosphere, or absorption of moisture.

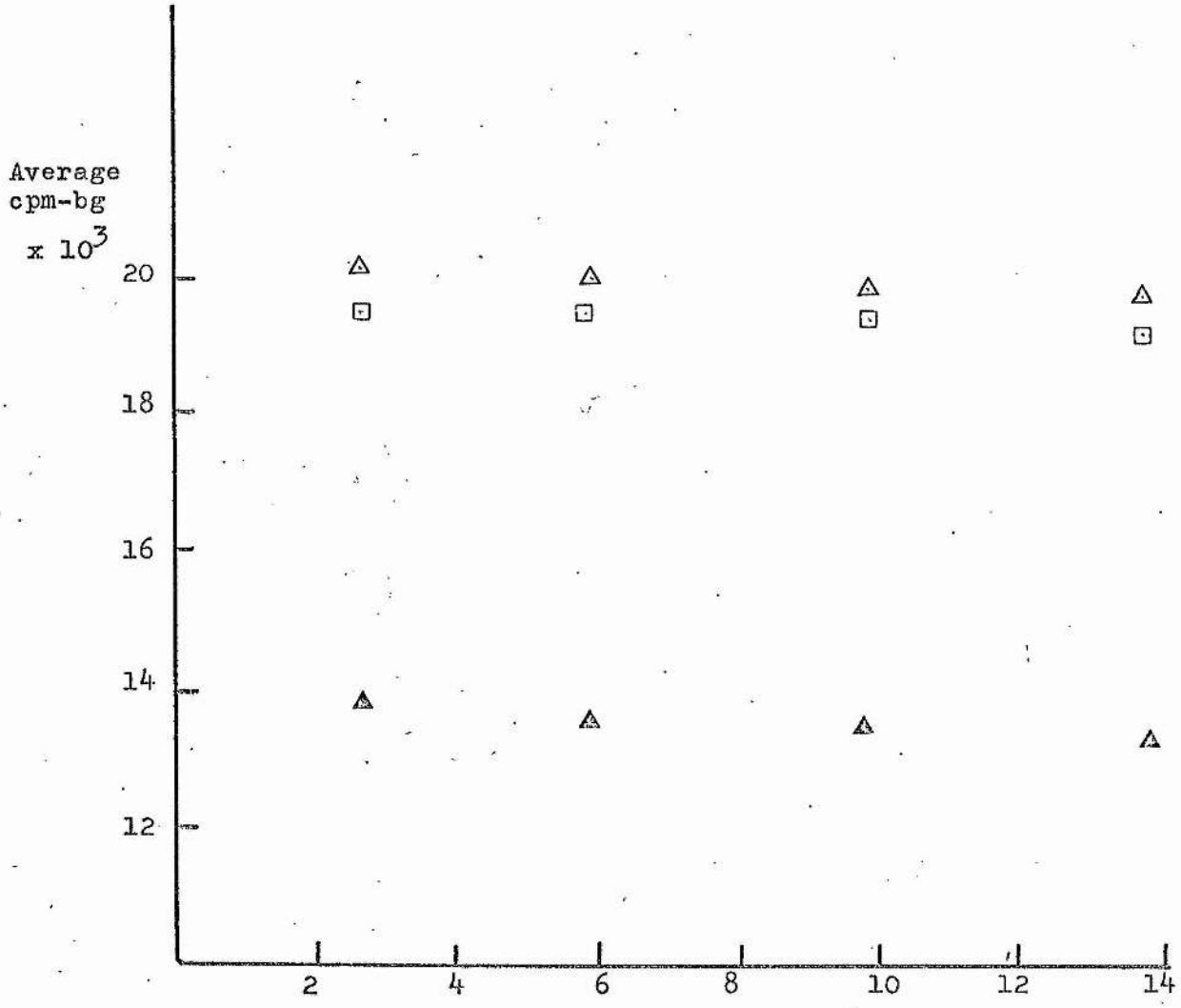


Figure 2.13

The average change in measured radioactivity with time of the three sets of planchettes (Lens tissue = △, Glass fibre = □, and direct = ▲.) .

2.6 Preparation of Plant Material for Counting

At the end of an experiment, the lights were switched off, and the plant material removed from the enclosure. It was washed quickly several times, in distilled water, to remove any traces of radioactive incubating medium and blotted dry. When discs or small leaves were used these were stuck directly onto the planchettes. If larger whole leaves were used, then discs were cut out of them, and stuck onto planchettes.

In the case of discs, the area of tissue involved is easily determined by the size of the cutter used. Whole leaves must, however, be measured. The procedure initially adopted was to trace the area of each leaf onto graph paper, before sticking it onto a planchette. The area of the graph paper was measured by counting the squares, weighing the cut-out area, or measuring the outline with a planimeter. The whole procedure was too time consuming in the multi-leaf experiments and the delay was too great before the leaves could be heat killed on the planchettes. So the leaves were first stuck to the planchettes and their areas measured after drying, directly on the planchettes using a planimeter. A perspex board was used for this, the planchette being fixed in position with double-sided sellotape. Areas could be measured to within 0·1 cm^2 and because of its convenience, this method was adopted.

Two methods for sticking the plant material flat onto the planchettes were considered. Either a drop of glycerine albumen was used as a glue or double-sided sellotape was stuck to the planchette first and the plant material stuck onto it. The following investigation was performed to determine the relative merits of the two methods.

The effect of using glycerine albumen or double-sided sellotape for sticking plant material to planchettes, on the measured radioactivity

Experimental A shoot of P. perfoliatus from plants grown in the greenhouse, after collection from L. Croispol, was sealed in a 1·8 l Kilner jar with the water it had been growing in. Twenty μCi of ^{14}C sodium bicarbonate were injected through the seal in the lid, and the jar was fixed horizontally to the platform of a metabolic shaker. A 150-watt lamp was used to provide illumination from above and the shaker was started. After 24 hours the shoot was removed and washed several times in distilled water. Sets of 11 mm diameter discs were cut from six leaves and stuck onto planchettes alternately using glycerine albumen or double-sided sellotape. All the planchettes were then heat-killed on a slide warmer and then covered with dilute acetic acid to remove any inorganic carbon present. When the acetic acid had dried off, the planchettes were counted for the ^{14}C present.

Results The average cpm-bg for the two sets of planchettes are given in Table 2.14, along with their standard errors. From this the standard deviation of the difference

Table 2.14

The effect of using glycerine albumen or double sided sellotape, to stick plant material onto a planchette, on the resultant count. The standard errors of the means of the two methods are given. The difference between the means and the standard error of the difference of the means are also given.

	Method of adhesion to planchettes	
	Sellotape	Glycerine albumen
Number of observations.	13	13
Average cpm-bg.	4309	4223
Standard deviation of the means.	169.2	133.4
Difference of the means.	86	
Standard deviation of the difference of the means.	215.5	

of the means is 2·5 times the measured difference of the means, and a null hypothesis tested with the 't' test was shown to be true. Thus, the two means are from the same population of discs, which would be expected if there was no difference in efficiency of counting between the two methods of mounting the discs on the planchettes. Therefore, the glycerine albumen does not appear to permeate the leaf tissue on the planchette and cause increased absorption of the radioactivity. The double-sided sellotape method was adopted as it was more convenient and allowed the planchettes to be weighed with the sellotape, prior to fixing the plant tissue to them. After they were dried they could be reweighed and a value for the dry weight of the tissue obtained. When planchettes, containing plant tissues, were flooded with dilute acetic acid to remove any unfixed inorganic ^{14}C present, a green coloration outside the area occupied by the leaf tissue was sometimes produced. This indicated that some of the contents of the leaf were mobilised and the counting geometry of the tissue altered. To try and overcome this, acetic acid was added carefully so that the area occupied by the plant tissue was just covered. The problem of the effectiveness of an acetic acid addition in removing inorganic ^{14}C and its possible effects on the self absorption of the tissue was investigated.

<u>The effect of repeated flooding of plant tissues with dilute acetic acid on the measured radioactivity.</u>

The set of planchettes from the previous experiment

was counted before any additions of dilute acetic acid. They were then returned to the slide tray heater and the plant tissues just covered with acetic acid. When this had dried off, they were recounted. This process was repeated twice.

Results The average change, for the 30 planchettes, with each addition of acetic acid is given as a percentage of initial activity remaining, in Table 2.15. The percentage loss of activity at each addition is also given. The first flooding can be seen to be the most effective, reducing the count by nearly 7%. The second flooding reduced the count by a still significant amount of nearly 4·5%, but the third addition had less than a 2% effect.

The acetic acid may also reduce the counts, by combining with the plant material, causing increased absorption. Shrinking of the plant material, caused by repeated heating, may also cause increased absorption. Redeposition of plant material over a larger area than that occupied by the leaf would increase the counts observed, but this could be eliminated by careful addition of acetic acid.

Therefore, it was assumed that the main effect of the acetic acid additions was to remove inorganic ^{14}C, and it was concluded that two additions of acetic acid performed this most efficiently. This procedure was then adopted as a standard technique.

Table 2.15

The effect of repeated flooding of the plant tissue, stuck to a planchette, on the measured radioactivity. The remaining radioactivity after each addition is expressed as a percentage of the initial radioactivity measured before addition of acetic acid. The percentage loss, of the initial radioactivity, at each addition is also given.

Number of additions of acetic acid.	Percentage of initial activity remaining.	Percentage loss at each addition.
0	100	0
1	93.2 ± 0.9	6.85
2	89.0 ± 1.4	4.46
3	87.5 ± 1.4	1.69

2.7 The Counting Procedure and Correction for Self Absorption

The radioactivity present in all the plant tissues and carbonate precipitates on the planchettes was counted on a Tracerlab automatic counter. The detector used was a gas flow counter with a thin foil end window to which the planchettes were presented.

Each set of planchettes from an experiment was loaded in carrier trays on the conveyor belt, along with background planchettes and the standard planchette. This was a polycarbonate polymer labelled with ^{14}C, which will give a constant count rate, and it was used to check for any changes in counting efficiency. These could be caused by temperature and gas flow irregularities, and if necessary the counts for a set of planchettes could be corrected for changes observed in the count of the standard planchette.

The planchettes were generally either counted six times for ten minutes or three times for 20 minutes. The standard error for the counting of planchettes was always significantly lower than any of the experimental errors. The average background count was always subtracted from the average count for each planchette.

All planchettes were weighed before and after a dried precipitate or plant material was added to them. The difference in weight gave the dry matter present and by measuring the area occupied by a sample the

weight per unit area was calculated.

The purpose of counting the radioactivity present in the tissue samples is to measure the amount of ^{14}C incorporated. Knowing the $^{14}C/^{12}C$ ratio, then the total carbon incorporated into the tissues may be estimated. The ratio $^{14}C/^{12}C$ is referred to as the specific activity of the isotope, and is usually measured in mCi/mmol. The specific activity of the $NaH^{14}CO_3$ supplied by the Radiochemical Centre at Amersham is known, but opening the sealed glass ampoule supplied and exposure to the atmosphere during dispensing and storage operations will cause this to be unknown. Similarly, the use of several dilutions and the additions of small amounts of a stock solution to an incubating solution will introduce further errors.

It is, therefore, necessary to measure the specific activity of each incubating solution used. The precipitates of the incubating solution, containing all the carbon in a known volume, were used for this. The concentration of total carbon in a solution was either known in the case of a bicarbonate solution or measured by titration when loch waters were used. The radioactive count for this known amount of carbon is then determined, and if the method is 100% efficient, all the ^{14}C beta-particles emitted by the sample in a given time will have been counted. Given the half-life of ^{14}C as 5,760 years (Wilson, 1966) then the total counts from a sample in a given time will be proportional to the

amount of ^{14}C present. Then the specific activity of the sample will be known.

However, the counter is not 100% efficient, and there are many factors which affect this. The use of an empirical standard, i.e. the standard polycarbonate planchette, as recommended by Wilson (1966), enables the efficiency of the counter to be measured. But this is the efficiency of the counter itself, which will be constant, and will not affect relative specific activity, nor the counting efficiency when using actual samples. This will depend upon the nature of each sample itself.

The interaction of ^{14}C beta-particles with matter must therefore be considered. Atoms of ^{14}C emit beta-particles which are not monochromatic but have a maximum energy of 0·159 Mev (Wilson, 1966) and an energy distribution similar to Figure 2.16 (Kamen, 1957). Beta-particles of this maximum energy are considered to be weak or soft (Libby, 1947) and there is an appreciable amount of absorption and scattering within the sample itself (Hendler, 1959). Beta-particles lose energy to absorbers by a series of interactions with orbital electrons, each of which robs it of a fraction of its energy. At each collision the momentum of each beta-particle may be altered and consequently its direction of motion can also be changed. This is more likely to happen when the energy of the beta-particle is low. This effect is responsible for the scattering of beta-particles as they pass through matter. Both the

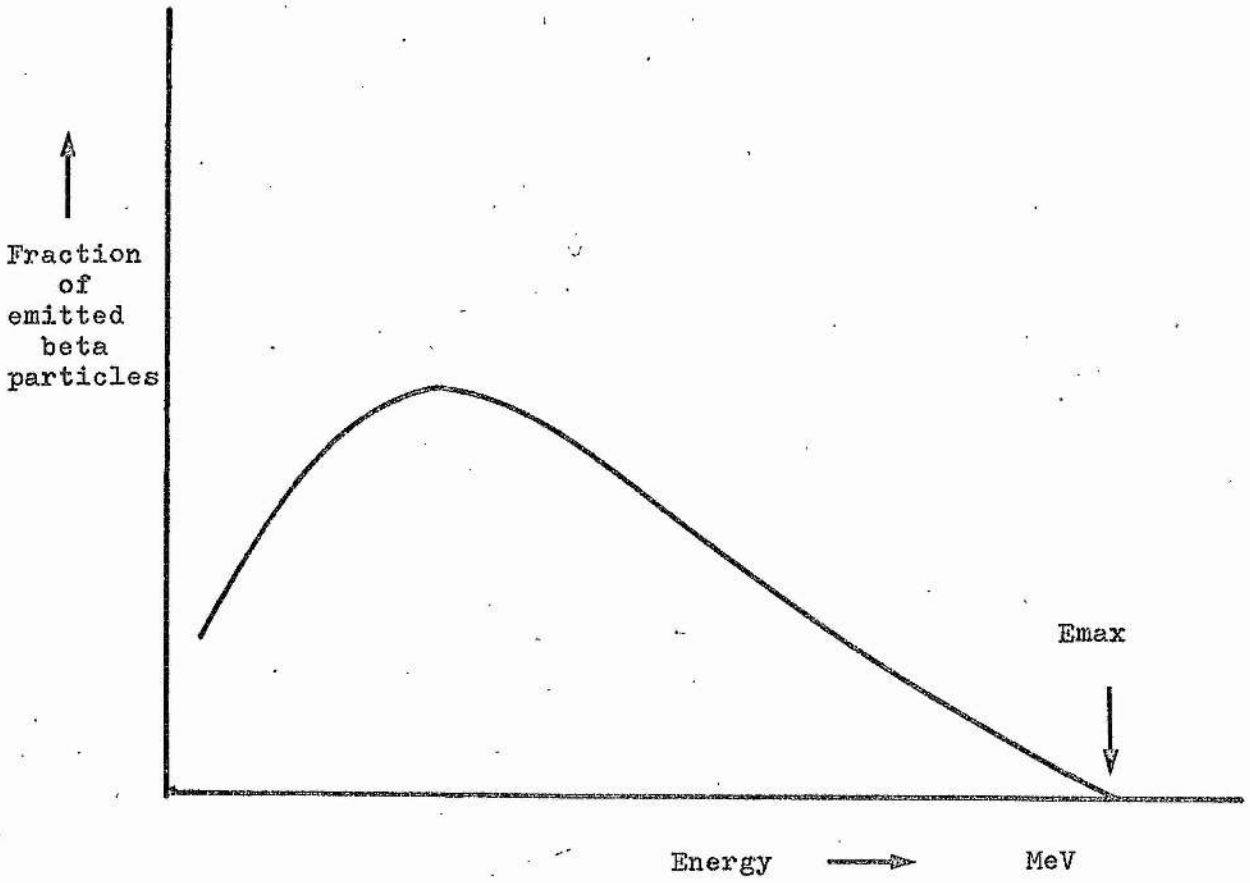


Figure 2.16

A typical beta ray spectrum. After Kamen (1957).

absorbing and scattering effects of matter will be a function of the density of the orbital electrons in the absorber. Generally, this is assumed to be proportional to the density of the absorbing material.

Previous workers have tended to consider that biological material can be counted assuming negligible absorption (Sorokkin, 1962). This has been with samples of unicellular algae, which can be filtered for counting and the amount per unit area can be controlled, thus keeping self-absorption to a minimum. However, this is not the case when using the leaves of aquatic macrophytes stuck directly onto planchettes. Alternatively, the leaf could be dried and ground to a powder and spread thinly on a planchette. But the hazards of handling a dry radioactive powder, the errors involved in transferring the sample completely to the planchette, and the problem of spreading the powder evenly over the planchette, indicated the method would not be suitable. Also it is possible to extract alcohol soluble ^{14}C from plant material and evaporate an aliquot of this extract to dryness on a planchette for counting. However, the uncertainty over the percentage of incorporated ^{14}C that is removed and the time taken to process many samples make this method undesirable.

The advantage of the method of sticking leaves directly onto the planchettes is one of ease of use and replication in the field.

Single leaves of aquatic macrophytes have been counted, when dried and stuck to a planchette, assuming negligible self-absorption (Spence and Campbell, 1971; Campbell, 1972; Black, 1972). To test this assumption for the plant leaves used in this study the following investigation was undertaken.

Self Absorption in Plant Leaves on Planchettes

Experimental A further 4 leaves were taken from the shoot of P. perfoliatus incubated in a previous experiment (Table 2.7) and discs cut from them in the same manner. Discs from any one leaf were stuck on top of each other in two's and three's as well as singly. These combinations were dried and counted as usual.

Results The counts obtaine for each planchette were divided by the number of discs on that planchette to give an average cpm-bg for each disc and are given in Table 2.17.

This shows that when two discs cut from a leaf are counted on top of the other the average cpm-bg per disc is always less than the average for the remaining discs counted singly (leaves G,I,J). Similarly, when three discs are placed on top of each other the average cpm-bg is even more reduced than with two discs on top of each other (leaf H).

Discussion These observations indicate that absorption of ^{14}C beta-particles by one leaf thickness is significant. Thus, in a planchette with two or more discs

Table 2.17

The effect of self absorption in leaf tissues as shown by the average cpm-bg per disc obtained for planchettes with one, two, or three leaf discs stuck on top of each other.

Number of discs on the planchette.	Leaf.			
	G	H	I	J
1.	3246	4064	3733	4762
2.	2342	-	2776	4426
3.	-	1825	-	-

self-absorption is taking place to a significant extent and this effect cannot be excluded from occurring significantly in a single disc.

The self absorption in the leaf tissues and the precipitates would have to be taken into account when calculating the amount of ^{14}C incorporation. Several methods were then considered for correction of self absorption in the leaf tissues and precipitates.

1. Count all Samples at Constant Thickness

If all the samples were carefully adjusted to be always of the same thickness then any correction factor for self-absorption would be constant. This would allow the counts for different samples to be compared, and if some of the samples could also be counted in the gas phase, at 100% efficiency, then the absolute specific activity could be calculated. This method, however, would be tedious to apply to the barium carbonate precipitate, reducing the advantages of this as a field technique. It would not be possible to apply it to the leaf tissue.

2. Count all Samples at Infinite Thinness

The self-absorption of a sample decreases as the mass per unit area decreases and if this becomes small enough, the self absorption can be considered to be negligible. However, the small amount of material would require a very high specific activity which is not always easy to obtain when incorporation is the result of a biological process. The thickness of the

leaf tissues is defined by the plant and has been shown to be thick enough to cause self-absorption (Table 2.17) and cannot easily be altered without processing the plant material. The low weights involved would introduce larger errors in estimating them.

3. Count all Samples at Infinite Thickness

The range of a beta-particle in matter will be limited and for a given isotope this can be calculated. If the sample thickness is larger than this range then the measured count rate will be proportional to the specific activity of the sample rather than the total radioactivity present. This method could be employed for the precipitates but not for the plant material as the leaf thickness used is less than this range.

4. Use an Empirically Determined Curve

A series of standard planchettes of increasing weight per unit area can be made up from a large homogenous sample of the material. If the range of weights per unit area covered by these is sufficient and less than the infinite thickness then a graph may be drawn up of cpm-bg against thickness of sample in $mg.cm^{-2}$. This graph can then be extrapolated to infinite thinness and the true cpm-bg at zero self-absorption can be estimated. The correction factor for low sample weights would be very difficult to determine accurately because of the increase in percentage uncertainty of the weight per unit area as the weight decreased to zero. Thus, the extrapolation to true cpm-bg at infinite thickness

becomes uncertain and the accuracy of this empirical curve is dependent upon the extrapolation. Although this curve is easily prepared for barium carbonate precipitates it would be more difficult to apply it in the case of the leaf tissues.

5. Use a Theoretical Absorption Curve

The assumption that the absorption of beta-particles from ^{14}C will be proportional to the density of the orbital electrons of the absorbing matter, i.e. $mg.cm^{-2}$ of the sample itself, allows a theoretical equation to be derived. The combination of the indeterminate absorption and scattering of ^{14}C beta-particles with their complicated energy spectrum (Figure 2.16) gives rise to experimental absorption curves which are very nearly exponential in form (Libby, 1947; Henriques, Kistiakowsky, Margnett, and Schneider 1946).

A calculated absorption correction for beta sources given by Wilson (1966) is shown in Figure 2.18. This nomogram for elements of medium atomic number is given as a useful practical guide and is not recommended for use where accuracy of less than 10% is required. Faires and Parkes (1960) give a formula for self-absorption of an isotope homogenously distributed in an absorbing medium as follows:

$$\frac{n_t}{n_o} = \frac{0.693 \cdot \frac{x}{d1/2}}{1 - (\frac{1}{2})^{\frac{x}{d1/2}}} \quad \ldots\ldots\ldots \quad 1$$

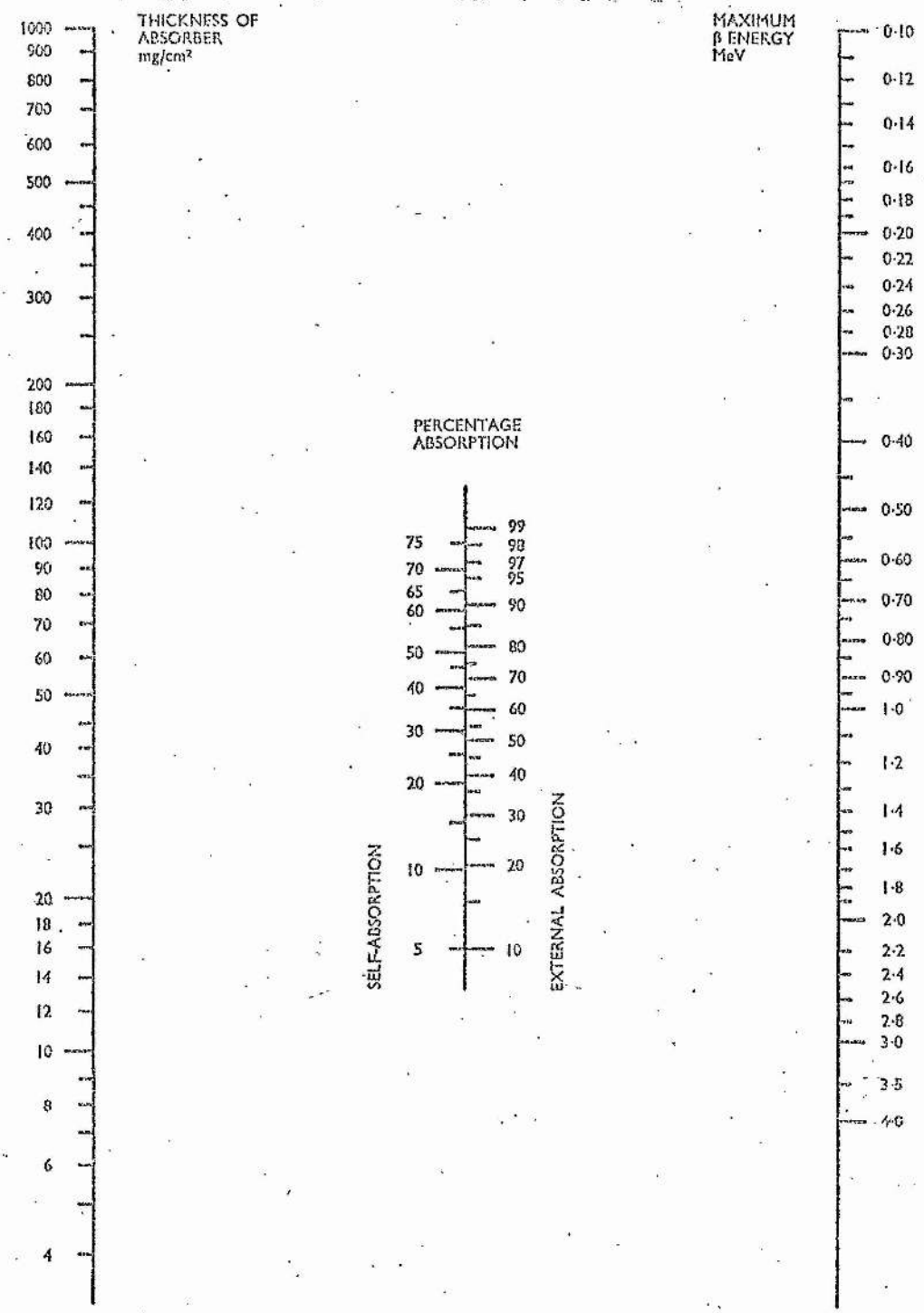


Figure 2.18

A nomogram for correction of self absorption of beta sources. From "The Radiochemical Manual" edited by Wilson (1966).

where n_t = true cpm

n_o = observed cpm

x = sample thickness in mg cm^{-2}

$d1/2$ = thickness in mg cm^{-2} that will stop half of the beta-particles of maximum energy E_{max}

d1/2 is given by (Cook and Duncan, 1952)

$$d_{1/2} = 45\ E_{max}^{1\cdot5} \qquad \text{.....} \quad 2$$

where E_{max} = maximum energy in Mev.

The value of E_{max} = 0·156 Mev for ^{14}C beta-particles can then be substituted into equation 2 to give $d_{1/2}$ of 2·77 mg cm^{-2}. This value when substituted into equation 1 allows the ratio of n_t/n_o to be expressed as a function of x, the sample thickness in mg cm^{-2}. The solutions of this function for $0 < x > 150$ mg cm^{-2}, $0 < x > 15$ mg cm^{-2}, and $0 < x > 1{\cdot}5$ mg cm^{-2} are given in Figures 2.19, 2.20, and 2.21, respectively.

These solutions were adopted in this study for the correction of self absorption in the tissue and precipitate samples. The use of a graph, however, is more tedious and introduces additional errors, than calculating n_t/n_o for the value of x, measured for the sample in question.

As the averages and background subtractions were computed on a 'Wang' card-programmable calculator, a program for calculation of self-absorption correction ratio from equation 1 was written. Therefore, all

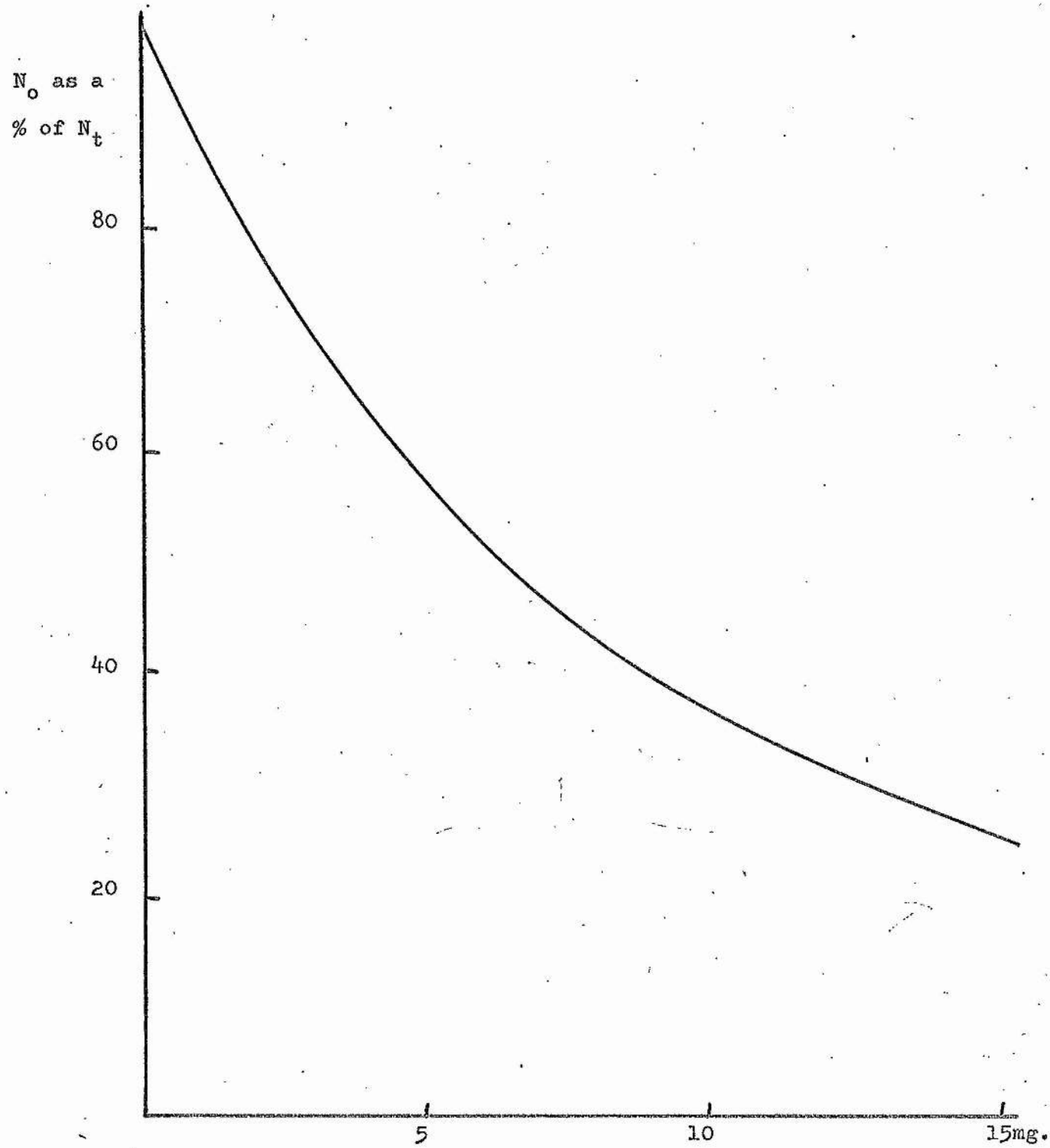


Figure 2.19

Self absorption correction for ^{14}C beta.

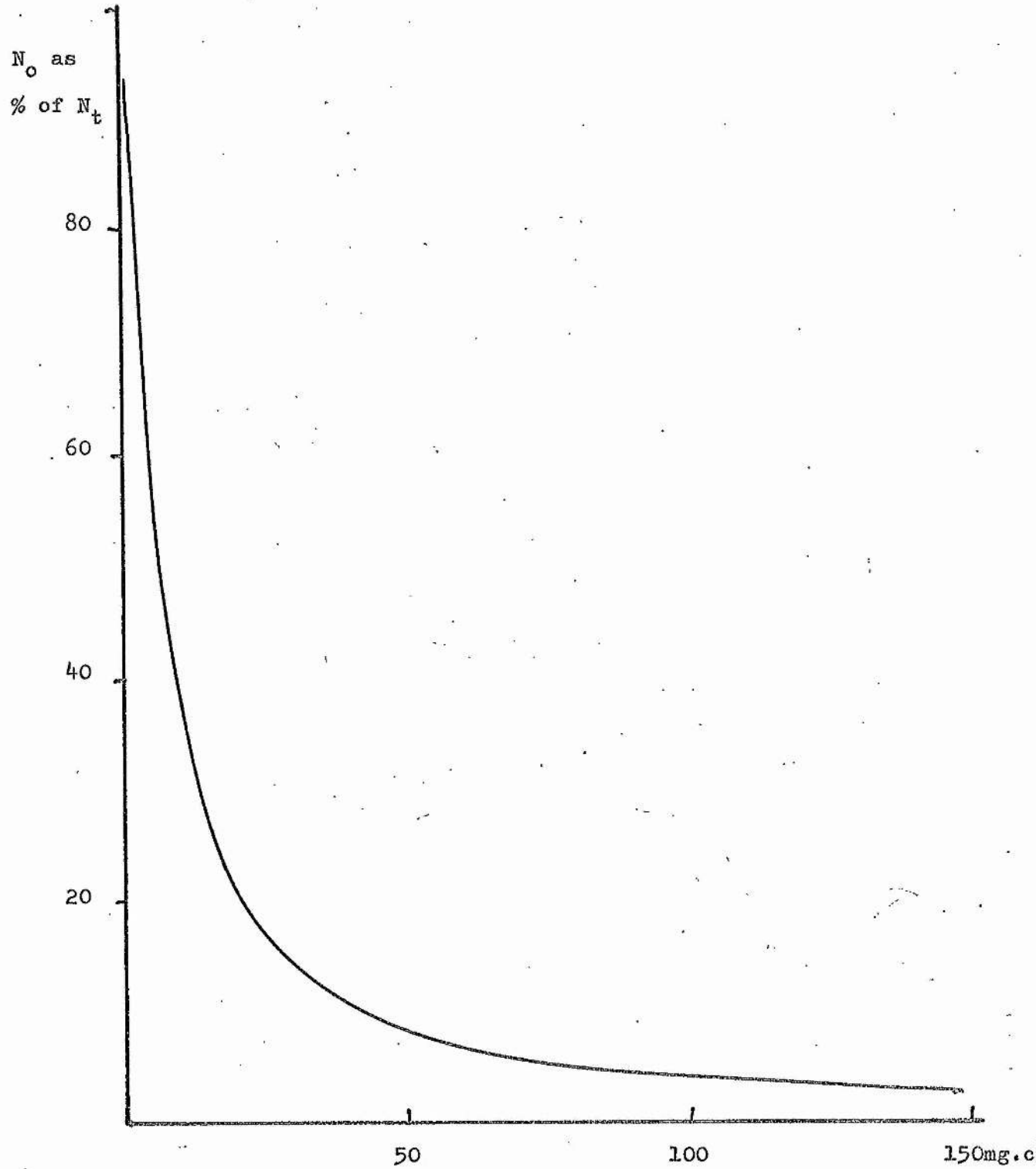


Figure 2.20

Self absorption correction for ^{14}C beta.

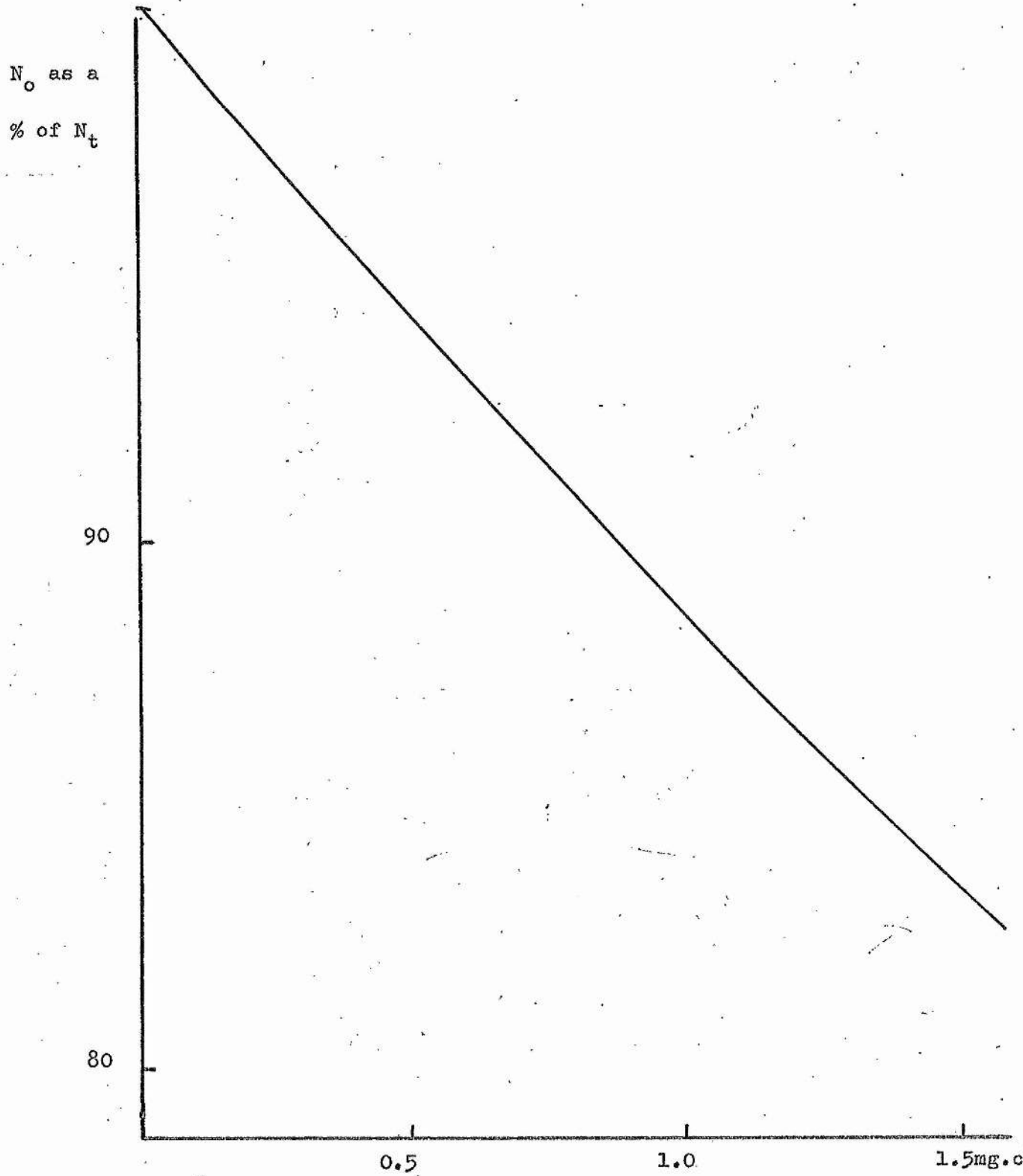


Figure 2.21

Self absorption correction for ^{14}C beta

samples were automatically corrected for self-absorption during the calculation of the results of an experiment.

As the geometry of the counter was constant for all the samples, it was not necessary to know the absolute specific activity, and the relative specific activity was used. That is, the ratio of cpm-bg per mole of carbon, measured from the precipitate, was applied directly to the cpm-bg for the plant tissue to get the moles of carbon incorporated.

However, the use of the relative specific activity uses the same assumption as the theoretical self-absorption correction method. That is, that the precipitates and tissue can be counted for ^{14}C beta with the same efficiency. This means they must have the same geometry with regard to the interaction of ^{14}C beta-particles with matter.

Unlike the barium carbonate precipitate, the intact plant material cannot be considered as homogenous. The cellular organisation and leaf veins cause the thickness of the leaf, stuck on the planchette, to be different and dependant on the position of the measurement. Thus, the weight per unit area is only an average value and the thicker parts will have more absorption and the thinner parts less. Thus, the average self-absorption may not correspond with the average weight per unit area. It is also unlikely that the ^{14}C incorporated into the

leaf tissues will be distributed homogenously and further complications with the averaging of self-absorption corrections will occur. Libby (1957) found that the rough surface of a crystalline powder required a larger correction for self-absorption geometry than a smooth surface particularly for soft beta radiation.

The back-scattering of ^{14}C beta from the aluminium planchette will be similar for both tissues and precipitates, but as the energy of the back-scattered radiation will be less than the original (Libby, 1957) i.e. softer, it will be more readily absorbed and thicker samples will show less contribution from back-scattering. The converse, that thinner samples will show a proportionately greater increase of back-scatter from the aluminium planchettes also applies.

The back-scatter caused by the sample itself, that is the self-scatter, is dependant upon the nature of the material itself. It has been shown (Steinberg and Udenfriend, 1957) that the back-scatter and self-scatter may vary significantly with material, i.e.

Colloidal film	100%
Aluminium	120%
Steel	178%
Lead	190%

They further showed that in the case of barium carbonate plated on aluminium the percentage of back-scattered radiation actually increases with increasing sample thickness since ^{14}C beta reflect more strongly from

barium carbonate than they do from aluminium.

The assumption that absorption of beta-particles is proportional to the electron orbital density and hence to the weight per unit area of the sample (Faires and Parkes, 1960) is for elements of medium atomic number. The barium atoms, in the carbonate precipitates, can be considered as having high atomic number and may cause deviation from this assumption.

It has been found that true experimental absorption is not normally observed with ordinary and window-type counters (Libby, 1957), and Hendler (1959) found that the self-absorption correction for barium carbonate precipitates appears to follow a hyperbolic curve much more closely rather than an exponential one. He indicated that with a gas flow counter the exponential treatment could differ from the hyperbolic one by over 30%.

These facts indicate that the errors of the method adopted here are uncertain. It has been suggested (Goldman, 1968; Sorokin, 1962) that this may lead to serious errors in the estimation of ^{14}C incorporation. If this method is to be continued as a field and laboratory method it will be necessary to accurately determine the amount of ^{14}C in each sample. This could be done by preparing two empirical self-absorption curves, one for the plant tissue and one for the barium carbonate precipitate and calibrating

these against absolute activity of the samples used (Goldman, 1968).

2.8 The Duration of the Incubating Period for ^{14}C Photosynthesis Experiments

The duration of the incubation period for ^{14}C *in situ* photosynthesis experiments can be chosen by the experimenter but there are several factors which need to be taken into account.

If the chosen incubation period is short then errors involved, with stopping and starting many replicate enclosures, will increase and also the measured ^{14}C incorporation will be low, allowing errors in the measurement of ^{14}C to affect the result. Further, the measured rates will be used to extrapolate over much longer time periods and short incubation periods may not be representative.

Long incubation periods, while overcoming these problems, may be affected by fatigue of the excised plant tissue and also by depletion of nutrients, particularly carbon, from the fluid in the enclosure.

In situ incubation periods have tended to be for several hours but for the present study it was decided to reduce this to one hour, both for laboratory and field experiments. To ascertain that the leaves had attained steady rates and not suffered from tissue fatigue, in this time, the following investigation was undertaken.

The incorporation of ^{14}C by P. praelongus discs, in the light and dark, as affected by different periods of incubation

Experimental Fresh shoots of P. praelongus were collected in black plastic bags from L. Drumore and transported directly to the laboratory. They were stored in fresh loch water under shade in a greenhouse until required for experimentation. Six healthy looking leaves were selected and two replicate 2 cm diameter discs cut from each. After cutting the discs were kept in 2×10^{-3}M $KHCO_3$ away from direct light. A replicate set of discs was then placed in each of light and dark enclosures (Figure 2.22) such that the individual discs were held in a row at right angles to the direction of incident light. All air bubbles were removed from the system which was filled with fresh 2×10^{-3}M $KHCO_3$. The flow system was switched on, 50 μCi of ^{14}C were injected through the 'suba' seal and allowed to mix thoroughly before the lights were switched on to commence the incubation. The mixing took less than one minute and had previously been checked using dyes. The sample for determination of initial specific activity was drawn through the 'suba' seal by syringe. The temperature of the incubating medium was held at $18^{o}C$ and was the same as that of the loch water at the time of collection. Illumination was provided by the overhead light system (see section 4.3) and gave $3{\cdot}6 \times 10^{3}$ microeinsteins m^{-2} s^{-1}.

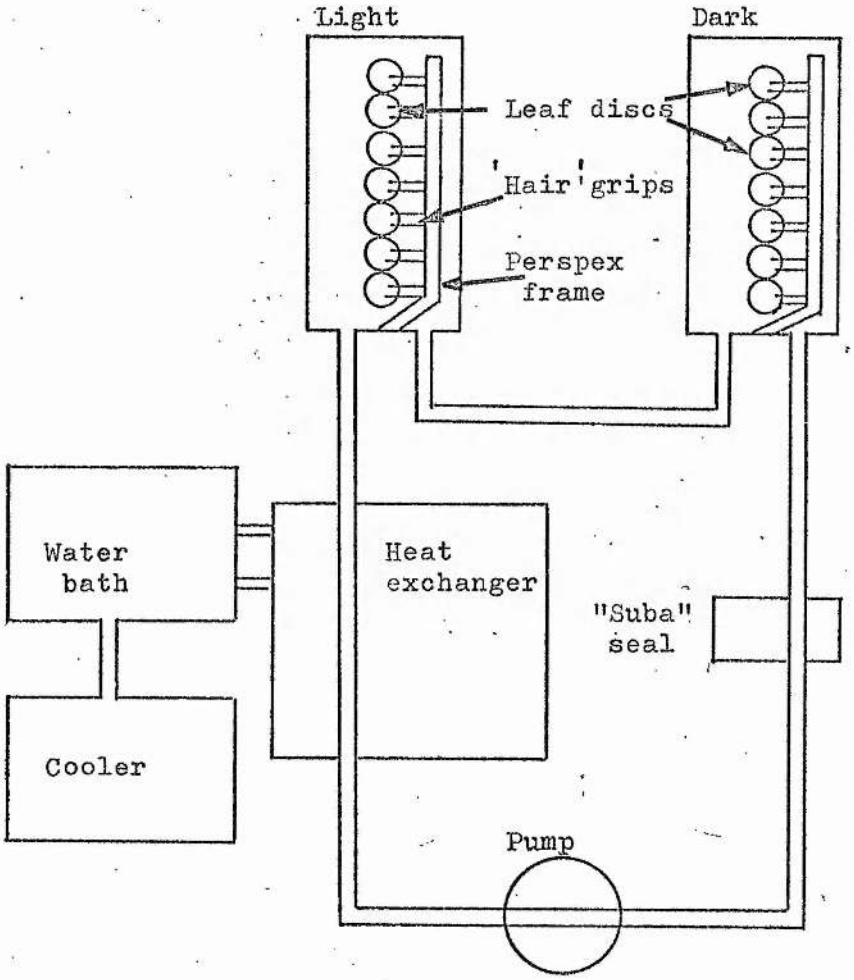


Figure 2.22

The light and dark 1.8 l kilner jars , used as enclosures, to measure the incorporation of ^{14}C by P.praelongus for different periods of incubation. The jars were laid on their sides and the illumination was from above. The light was prevented from entering the dark bottle by layers of black tape. The perspex frames inside each jar were used to hold the hair grips in a row so that the leaf discs were all held at the same height and prevented from being sucked around the system

At one minute before the chosen incubation period was due to finish a final sample for determination of specific activity was withdrawn. The lights were switched off at the end of the incubation period and the two sets of discs were removed from the radioactive solution as quickly as possible and rinsed in non-radioactive solution before heat-killing and preparation for counting. The experiment was repeated for different periods of incubation of: 10,20,40, and 60 minutes.

Results The discs were counted and corrected for self-absorption. The counts from each set of six discs were then averaged and expressed as the average carbon uptake per mg dry weight of tissue in the light or dark (Table 2.23a) for the different periods of incubation. These have been extrapolated to give the average carbon uptake per hour for each incubation period (Table 2.23b). The standard errors of the mean for each set of six discs are also given. From the data in Table 2.23 the carbon uptake as moles x 10^{-7} CO_2 per mg dry weight of tissue is plotted against the duration of the incubation period (Figure 2.24). This gives two plots, one for the light uptake, and one for the dark uptake.

Discussion The uptake of $^{14}CO_2$ in the light is remarkably linear with time. Assuming the leaves to be performing steady state photosynthesis then this would be expected. The dark uptake, however, is not so linear and it would not be expected to be active uptake but a

Table 2.23

The effect of varying the incubation period on the measured carbon uptake in the light and dark; a/ The average carbon uptake, as moles x 10^{-7} CO2/mg dry weight, in the light and dark for each period of incubation. b/ The rate of carbon uptake expressed as moles x 10^{-7} CO2/mg dry weight/hour extrappolated from each of the incubations in a/. The range of each figure is given as the standard error of the mean for the six replicate discs in each treatment.

a/ Duration of incubation period in minutes.

	10	20	40	60
Light.	1.89±0.32	4.63±0.47	9.29±0.90	14.1±0.75
Dark.	0.14±0.03	0.21±0.02	0.26±0.02	0.32±0.05

b/ Duration of incubation period in minutes.

	10	20	40	60
Light.	11.34±1.90	13.80±1.39	13.90±1.35	14.10±0.75
Dark.	0.84±0.19	0.63±0.06	0.39±0.04	0.32±0.05

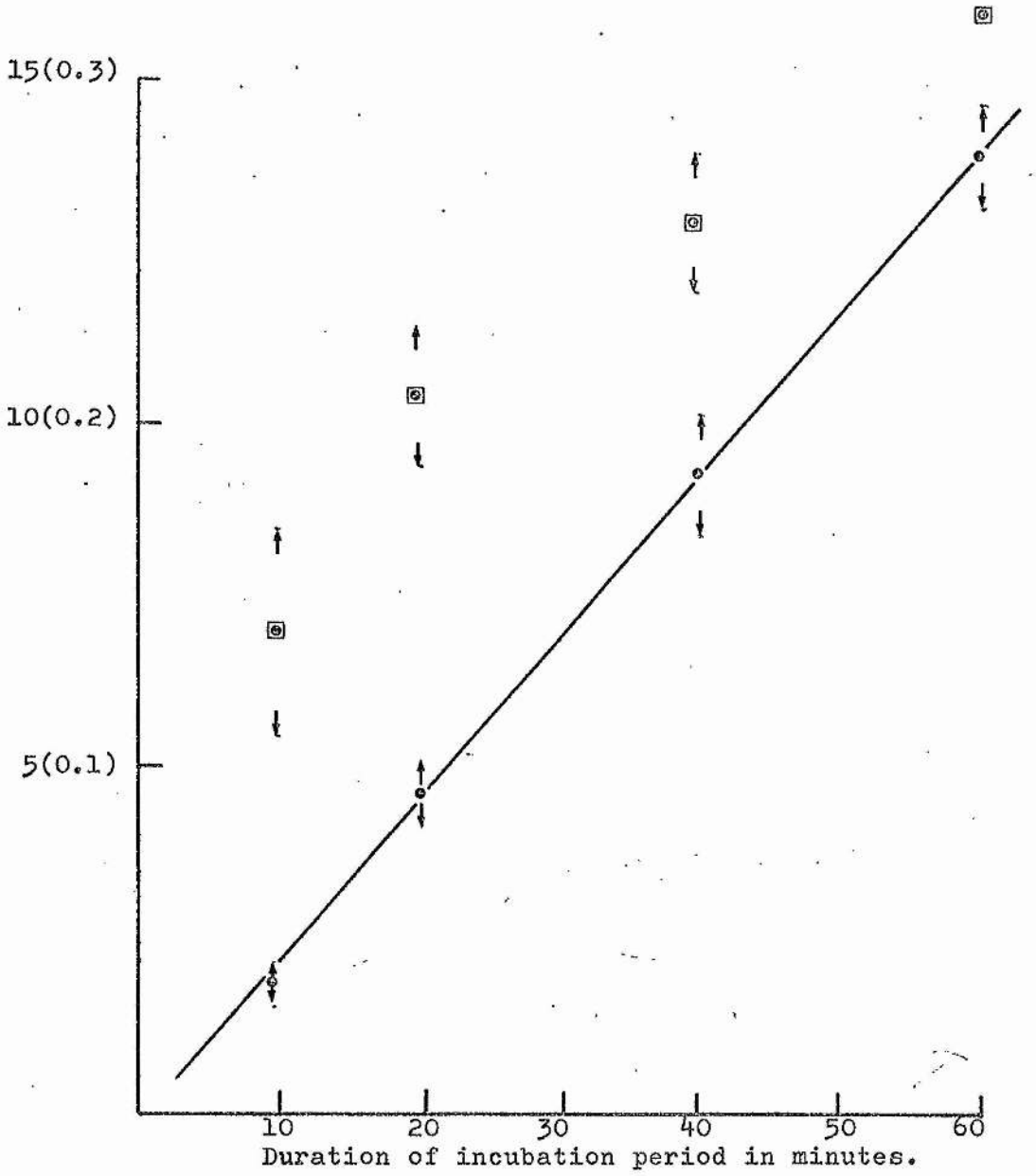


Figure 2.24

The mean carbon uptake plotted as moles x 10^{-7}/mg dry weight against the duration of incubation, from table 4.17 . The light uptake is denoted as • and the dark as ⊡ . The scale used for the dark uptake is larger and is shown in parentheses. The standard error of the mean of each plot is denoted by the limit of the arrows.

passive diffusion or equilibration of $^{14}CO_2$ with the internal compartments. It shows an initial rate which declines with time. A log-linear plot is given in Figure 2.25 of the dark uptake and shows two distinct phases of uptake. The first phase of 0-20 minutes may be controlled by the equilibration of one compartment with the external solution. This then ceases to be controlling as a second inner compartment starts to control the uptake over the 20 to 60 minute period.

Thus, over periods of 10 to 60 minutes it would appear to be valid to measure the light uptake of $^{14}CO_2$ but the dark uptake must be interpreted more carefully. After 10 minutes the dark uptake was 7·4% of the light uptake, but after 60 minutes it had decreased to 2·3%.

These results suggest that the choice of a one hour incubation period does not significantly incur any of errors of too long or too short an incubation period and it was therefore decided to adopt the one hour incubation period for subsequent ^{14}C incorporation experiments.

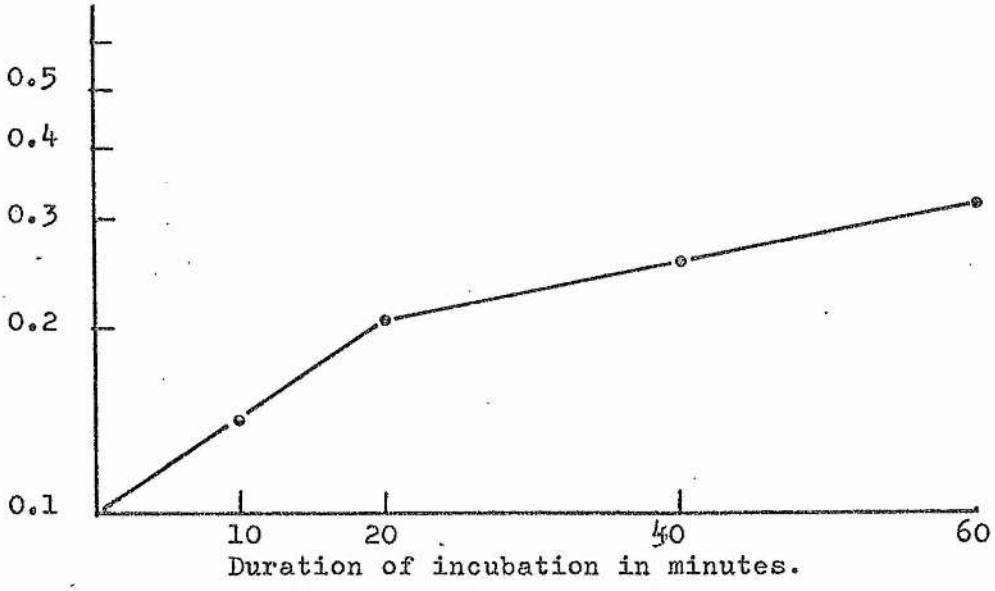


Figure 2.25

The carbon uptake in the dark as moles x 10^{-7}/mg dry weight, for discs of Potamogeton praelongus, expressed on a log scale against the duration of incubation.

2.9 The Rate of Release of Previously Fixed ^{14}C from Leaves of P. praelongus, in the Light and in the Dark

Interpretation of photosynthesis rates measured by the ^{14}C incorporation technique requires information about the fluxes of ^{14}C both into and out of the tissues. Unlike light and dark bottle oxygen measurements the ^{14}C dark enclosures cannot be used to estimate respiratory losses and hence enable calculations of gross photosynthesis to be made.

For long incubation periods, of the order of hours, photosynthesized ^{14}C will be respired and released as $^{14}CO_2$ from the tissues. The method will then approximate to net ^{14}C incorporation, that is net photosynthesis. However, as the incubation period is shortened then ^{14}C incorporation will approximate to gross photosynthesis.

Therefore, this experiment was conducted to investigate the rates at which photosynthesized ^{14}C is lost by leaves under conditions, of high light and high oxygen concentration, which would be expected to promote this loss.

Experimental Shoots of P. praelongus were collected fresh from Loch Drumore and stored in loch water under shade in the greenhouse prior to requirement in the experiment. Fresh looking leaves were selected and immersed in 2×10^{-3} M potassium bicarbonate solution

for a few minutes in the dark. The leaf was then sealed in a 30 ml Mcartney bottle with fresh bicarbonate solution. Twenty five μCi of ^{14}C bicarbonate were added by injection through the rubber seal in the cap and the bottle was incubated in the light for one hour. Illumination was provided from the side by a 150 watt lamp with a glass water tank positioned in between to reduce the heating effect, as in Figure 2.26. Precipitates of the incubating solution were taken at the beginning and at the end of the incubation. After one hour the leaf was removed quickly from the vessel and washed briefly in fresh non-radioactive bicarbonate solution before enclosure in the efflux chamber (Figure 2.26). This had the same volume as the Mcartney bottle and was placed in front of the same light regime.

This operation took less than 30 seconds to perform and the peristaltic pump was switched on immediately after sealing the leaf in the efflux chamber. This sucked fresh non-radioactive 2×10^{-3}M potassium bicarbonate solution, made up from oxygenated (10 ppm) distilled water, past the leaf at a rate of 20 ml per minute. At intervals of 5 minutes, counting from the end of the incubation period, a 3 ml sample of the efflux fluid was collected by syringe from the sample point and barium bicarbonate precipitates prepared from them. The waste collection chamber around the sample point was arranged so that the syringe needle could be placed directly into the end of the tubing and the sample

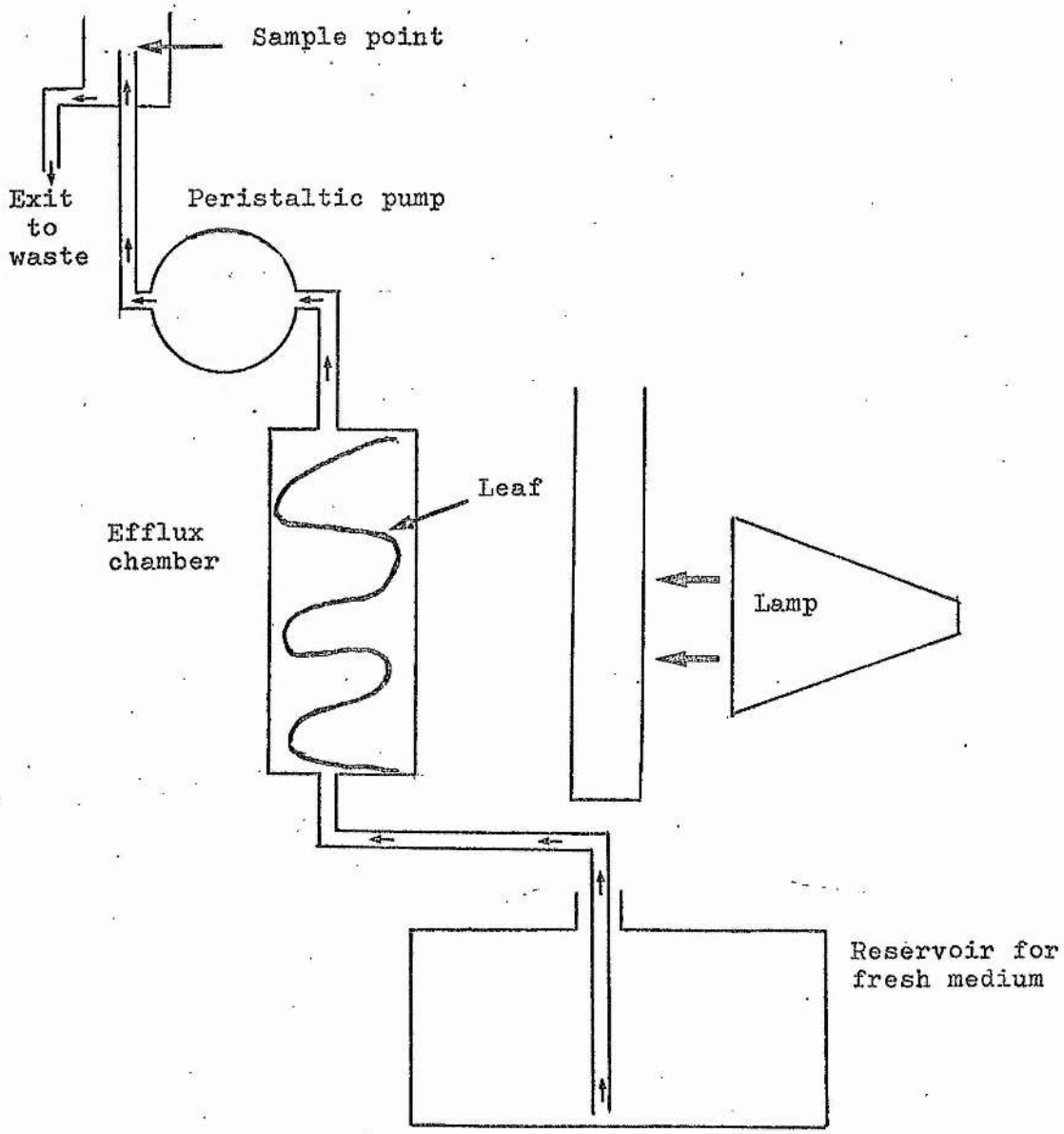


Figure 2.26

The experimental apparatus used to follow the rate of release of previously fixed ^{14}C from leaves of P. praelongus, in the light and in the dark.

drawn directly from the fluid flowing past the leaf at that time not from a pool of previously effluxed solution.

After 30 minutes of efflux under illumination, the light was switched off and the efflux chamber wrapped in several layers of black polythene sheet to ensure that no light reached the leaf. At the end of a further 30 minutes in the dark, the experiment was stopped, the leaf removed from the chamber, washed and heat-killed in preparation for counting. The precipitates and leaf were then counted in the usual manner.

The experiment was then repeated using similar leaves of P. praelongus. Additional experiments were performed with the dark period occurring for the first 30 minutes of the efflux period and the light period for the second 30 minutes.

Results The cumulative release of previously fixed ^{14}C against time is shown in Figure 2.27 and Figure 2.28. The ^{14}C incorporation by the leaves during the first hour of incubation was calculated by adding the total amount of ^{14}C released during the second hour to the amount of the ^{14}C remaining incorporated in the leaf after the end of the second hour. The total quantity of released ^{14}C was estimated by multiplying the sum of the ^{14}C counts for the precipitate by the ratio of the volume of the fluid collected to the volume of the fluid that flowed past the leaf during the second hour. The total release of ^{14}C during the second hour, for

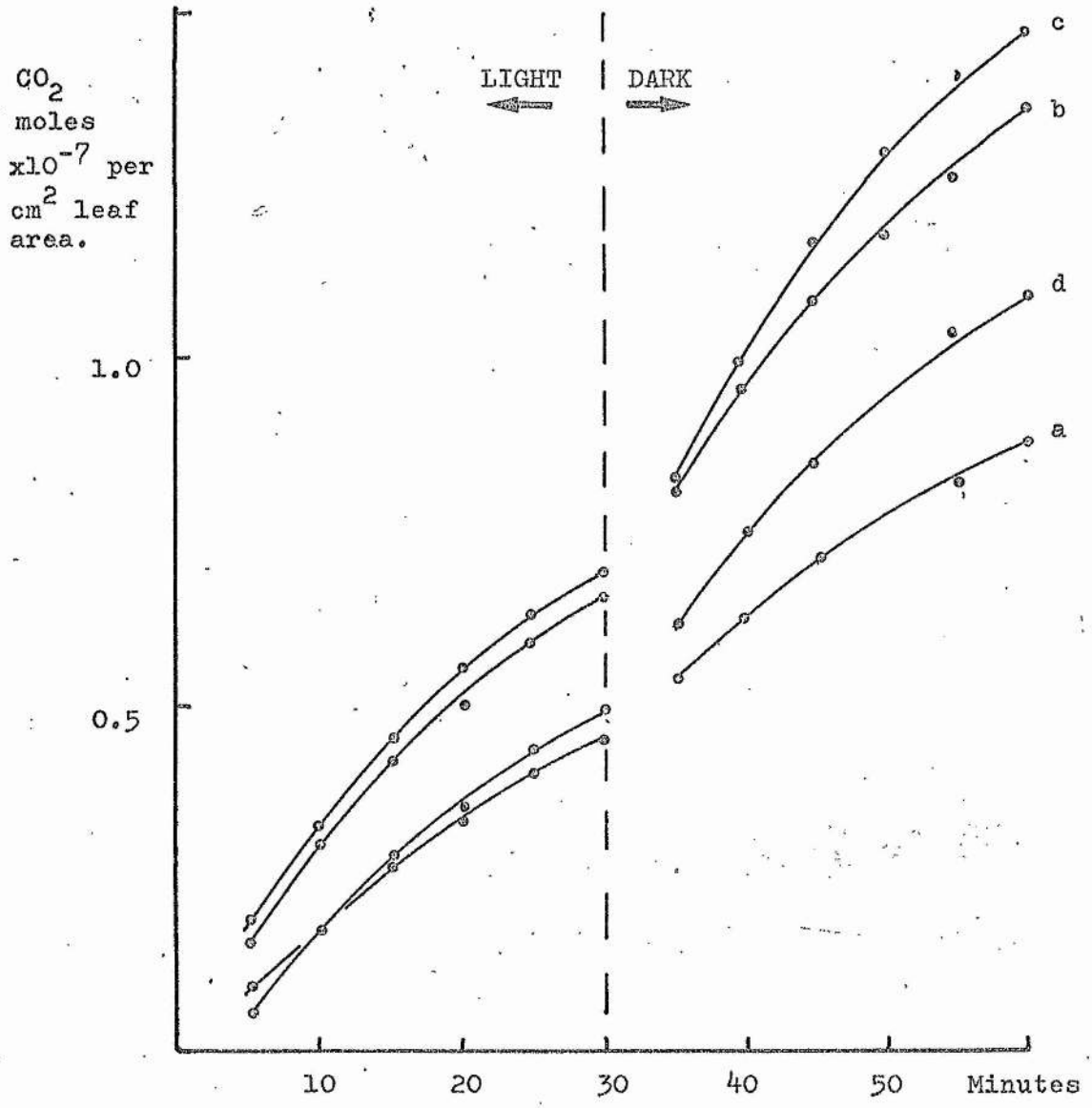


Figure 2.27

The cumulative release of ^{14}C, as moles x $10^{-7} cm^{-2}$ leaf area from individual leaves (a to d) previously incubated in ^{14}C bicarbonate solution for one hour. The leaves were illuminated for the first 30 minutes and kept in the dark for the second 30 minutes.

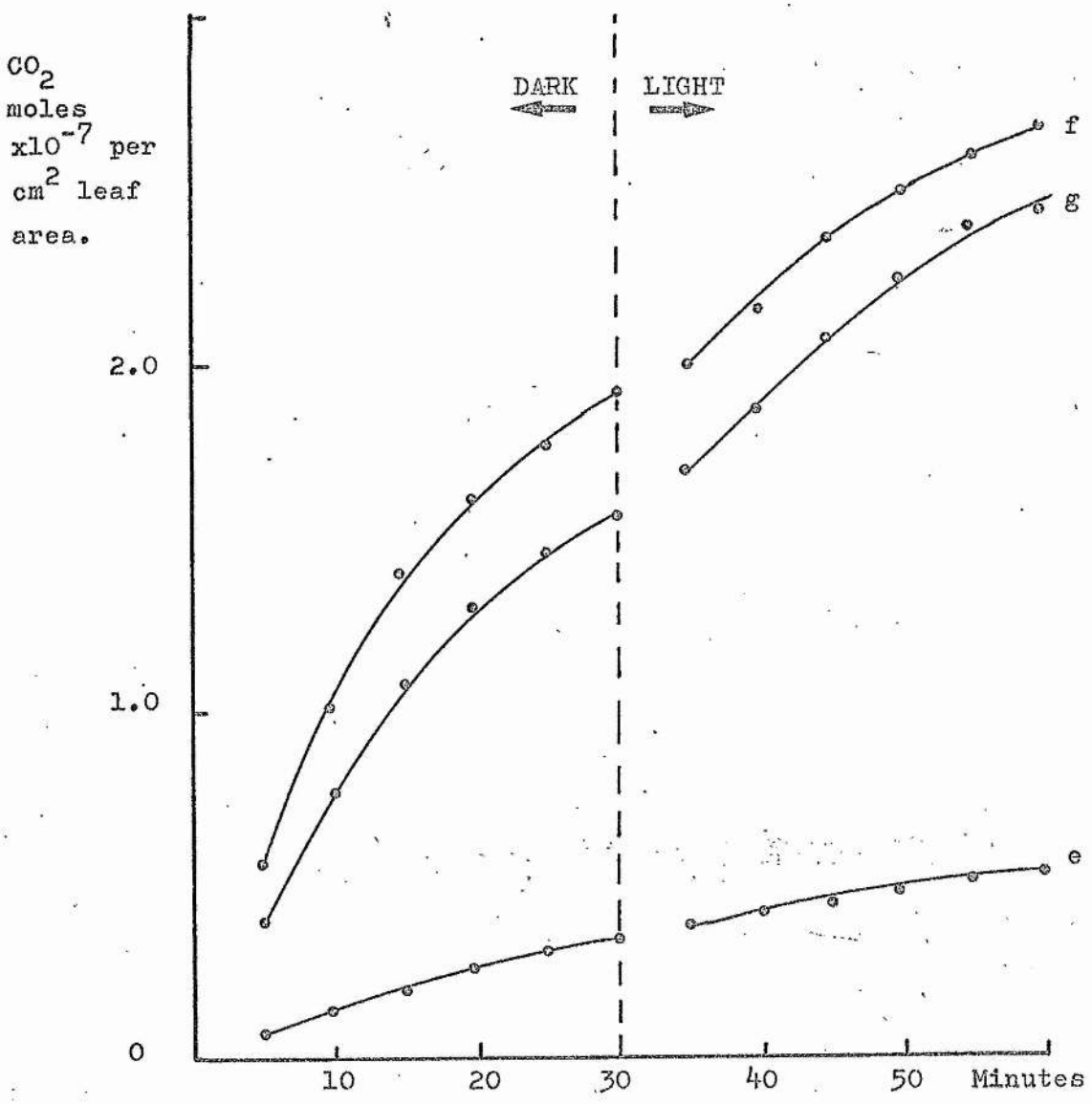


Figure 2.28

The cumulative release of ^{14}C, as moles x 10^{-7} cm^{-2} leaf area from individual leaves (e to g) previously incubated in ^{14}C bicarbonate solution for one hour. The leaves were illuminated for the first 30 minutes and kept in the dark for the second 30 minutes.

each leaf is given in Table 2.29 as moles x 10^{-7} CO_2 per cm^2 leaf area. The total ^{14}C incorporation for the first hour is given in the same manner and the ^{14}C release is expressed as a percentage of the ^{14}C incorporated. This is further expressed in Table 2.30a for the separate light and dark periods of the release and in Table 2.30b as the average release as a percentage of the total released.

Discussion The preliminary one hour incubation in the light with ^{14}C bicarbonate solution will allow the leaves to develop a ^{14}C-labelled cellular organic pool. Respiratory activities will cause some of this to be consumed and released as $^{14}CO_2$. Assuming that any extracellular $^{14}CO_2$ remaining from the preliminary incubation, is removed by washing or loss during the first 5 minutes of the release period, then the release of $^{14}CO_2$ during the second hour of incubation will be a measure of the release of ^{14}C from the cellular organic pool.

This is shown to be 20-40% of the total $^{14}CO_2$ incorporated in the previous hour (Table 2.29).

The specific activity of the ^{14}C-labelled carbon will change on its route from $^{14}CO_2$ (external), through photosynthesis, organic carbon pool and respiration to $^{14}CO_2$ (external) again. Assuming that there are no significant differences between the transport of ^{14}C and ^{12}C around this system, other than the isotopic effect due to mass of about 10% (Wilson, 1966) then the

Table 2.29

The amounts of ^{14}C, as moles x $10^{-7} cm^{-2}$ leaf area incorporated in one hour and released during the subsequent hour, for whole detached leaves of P. praelongus. The ^{14}C released is also expressed as a percentage of the amount of ^{14}C incorporated.

Leaf.	Incorporated ^{14}C	Released ^{14}C	Released as a % of incorporated
a	4.83	0.88	18.2
b	3.84	1.36	35.3
c	3.50	1.46	41.7
d	4.16	1.08	26.0
e	2.10	0.53	25.2
f	6.25	2.66	42.6
g	7.26	2.45	33.8

Table 2.30

The release of previously incorporated ^{14}C., in the light and dark, for the first 30 minutes and second 30 minutes of the release period.

a/ For each leaf (a to g) as a percentage of initially incorporated ^{14}C

Time period.	Light.	Dark.
0-30 minutes	9.07((a)	16.0 (e)
	17.1 (b)	30.2 (f)
	19.7 (c)	21.8 (g)
	11.6 (d)	
30-60 minutes	9.0 (e)	9.14 (a)
	12.4 (f)	18.2 (b)
	11.9 (g)	22.1 (c)
		14.3 (d)

b/ The average release as a percentage of the total released.

Time period.	Light.	Dark.
0-30 minutes.	47.5	69.7
30-60 minutes.	30.3	52.5

changes in specific activity will be caused by dilution. The specific activity of the labelled cellular organic carbon pool will be less than that of the $^{14}CO_2$ external to the leaf. During the first hour of incubation this pool will reach a plateau specific radioactivity which cannot be greater than that of the $^{14}CO_2$ supplied externally. This pool is the substrate for respiratory activities which will produce intracellular $^{14}CO_2$, some of which will be released to the external medium.

The comparison of the amounts of incorporated and released carbon depends upon the assumption that the specific activities will be the same. As the ^{14}C is diluted on its route through the plant, the release of CO_2 under steady state conditions will be underestimated. Thus, it is possible that the release of previously fixed CO_2 may significantly exceed 40% of that fixed in the previous hour under the conditions of the experiment.

During the release period there is no $^{14}CO_2$ being incorporated and there will be a steady decrease in the specific activity of the ^{14}C internal pools. This is shown by the decline in the rate of release of $^{14}CO_2$ with time (Figures 2.27 and 2.28) and extrapolation of the rate of release to the beginning of the release period will give a higher rate of release. That is the average release over the release period will be lower than the actual rate of release.

The rates of release of $^{14}CO_2$ in the light and the dark (Table 4.24) show that each leaf releases more previously fixed $^{14}CO_2$ in the dark than in the light. This is true whether the light period followed the dark or vice versa. This will be caused by re-incorporation of released $^{14}CO_2$, by photosynthesis back into the cellular organic pool. However, the respiration in the dark and light must be considered. The conditions of the experiment, high light and high oxygen concentration would be expected to encourage photorespiration (Hough, 1974). Thus, assuming P. praelongus to be a C_3 - Calvin cycle plant the production of $^{14}CO_2$ in the light will be much greater than in the dark. That this is not released indicates that the refixation of $^{14}CO_2$ by photosynthesis occurs at a rate exceeding the production of $^{14}CO_2$ by photo-respiration.

Thus, the release of previously fixed $^{14}CO_2$ is not greater in the light than in the dark unlike the situation found in terrestrial Calvin cycle plants (Zelitch, 1971). This agrees with the observations of Wetzel and Hough (1973) for submerged aquatic plants.

These results indicate that ^{14}C incorporation experiments, on broad leaved pondweeds, will over-estimate net photosynthesis and underestimate gross photosynthesis.

2.10 The Incorporation of ^{14}C, Supplied to the Roots, by the Leaves on a Whole Plant of P. perfoliatus

The roots and stems of submerged aquatic plants consist of tissues where respiration will predominate. It is possible that the carbon dioxide released by this process may be able to find its way, via the lacunae, to some of the leaves. As this may represent an internal source of inorganic carbon for photosynthesis, that would not be available to an excised leaf, the following experiment was conducted to estimate the possible involvement of the root of a whole plant in supplying carbon dioxide to the leaves.

Experimental A healthy plant of P. perfoliatus was uprooted carefully from an artificial pond in the greenhouse and washed in 2×10^{-3}M $KHCO_3$. This was placed horizontally in a perspex box so that the root/shoot interface lay through the centre of the groove cut in the partition (Figure 2.31). The groove between the two compartments was packed with vaseline so that there could be no transfer of liquid from one compartment to the other. The two compartments were then filled with 2×10^{-3}M $KHCO_3$, and 100 μCi $NaH^{14}CO_3$ were added only to the root compartment and its contents mixed. The lid was placed on the apparatus which was placed below a bank of fluorescent lights in the same position that the plant had originally been growing in. The plant was left in this position under illumination for 24 hours. Samples were taken from the root chamber at

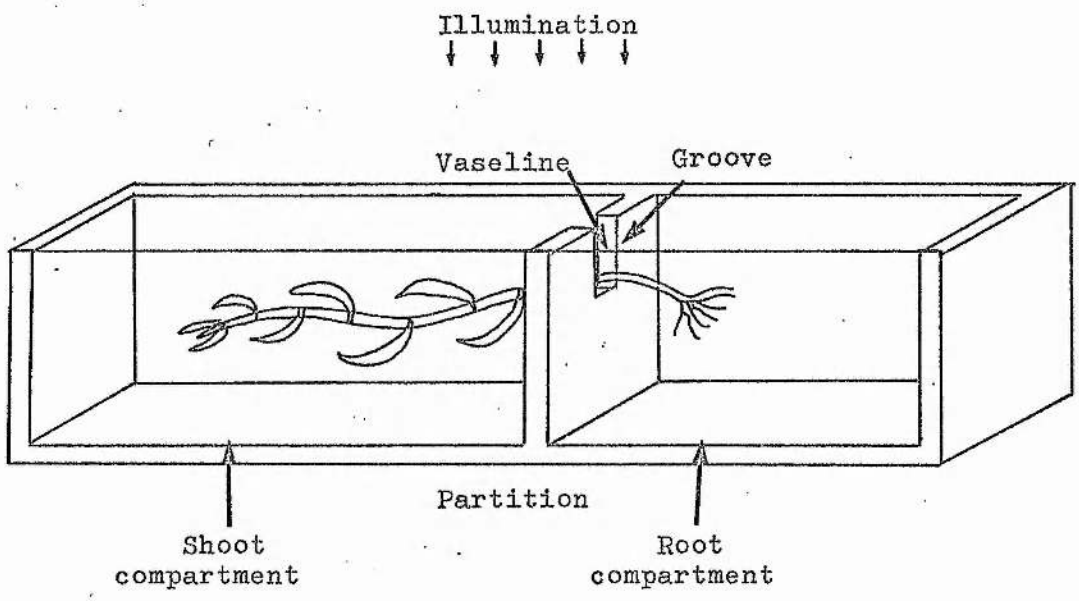


Figure 2.31

The chamber used to follow the incorporation of ^{14}C, from the roots to the shoot, of a whole plant of P. perfoliatus. The two compartments are separate and the interchange of liquid is prevented by packing the groove around the root/shoot interface with vaseline.

the beginning of this period and from both chambers at the end of this period. Precipitates were obtained from these and counted in the usual manner.

At the end of the incubation period the shoot was cut off at the root/shoot junction, and washed in distilled water. The leaves were removed, stuck onto planchettes and dried for counting. Their dry weights and areas were measured. The distance of the point of attachment of each leaf to the shoot was measured from the root/shoot interface.

Results The specific activity of the solution in the root compartment was calculated from the average of the initial and final precipitates. This was then used to estimate the incorporation of ^{14}C by the leaves on a leaf dry weight basis and a leaf area basis (Table 2.32). The specific activity of the solution surrounding the shoot was calculated from the precipitate taken at the end of the 24 hour period.

Discussion The measured rates of ^{14}C incorporation in the light for leaves of P. perfoliatus grown under similar conditions were found to be between 3·72 and 7·88 moles x 10^{-7} CO_2 per cm^2 per hour (Table 4.21). The maximum incorporation in this experiment occurred in leaf number 1, that is nearest to the root, and was 8·96 x 10^{-8} moles CO_2 per cm^2 for a 24 hour period. This would equal an average rate of 0·037 x 10^{-7} moles CO_2 per cm^2 per hour, which is approximately 1/2 to 1% of the measured incorporation rate from an external solution. As the distance of the point of attachment

Table 2.32

The incorporation of ^{14}C from the root to the shoot of a plant of P. perfoliatus. The results are expressed as the number of moles of CO_2 incorporated per cm^2 leaf area and per mg leaf dry weight for a 24 hour period. The distance of the point of attachment of each leaf to the shoot, measured from the root, is given along with its dry weight and surface area.

Leaf no.	Leaf dist. cm.	Leaf area cm^2	Leaf dry wt. mg	^{14}C incorporation per cm^2 area.	per mg dry wt.
1	0.7	0.7	1.1	8.96×10^{-8}	5.7×10^{-8}
2	1.7	1.7	2.2	2.48×10^{-8}	1.91×10^{-8}
3	4.1	2.4	2.5	5.72×10^{-9}	5.49×10^{-9}
4	6.9	2.4	3.0	2.57×10^{-9}	2.06×10^{-9}
5	10.2	2.4	2.6	1.30×10^{-9}	1.19×10^{-9}
6	12.9	2.2	2.4	9.30×10^{-10}	8.51×10^{-10}
7	15.5	1.9	2.3	1.06×10^{-9}	8.77×10^{-10}
8	17.2	1.8	2.0	8.51×10^{-10}	7.60×10^{-10}
9	18.3	1.6	2.2	8.00×10^{-10}	5.50×10^{-10}
10	18.9	1.6	2.0	1.13×10^{-9}	9.04×10^{-10}
11	19.2	2.1	2.3	7.80×10^{-10}	7.20×10^{-10}
12	19.5	2.3	1.8	8.77×10^{-10}	1.11×10^{-9}

of the leaf, from the root increases, the incorporation falls quickly with the first 4 leaves being responsible for most of the incorporation (94·1%).

The radioactive count measured in the shoot compartment indicates that $6{\cdot}92 \times 10^{-6}$ moles CO_2 were released into it. This assumes that the specific activity of the released carbon will be the same as that of the root compartment. This can be added to the amount of incorporated carbon in the twelve leaves ($1{\cdot}30 \times 10^{-7}$ moles CO_2) to give a total of $7{\cdot}05 \times 10^{-6}$ moles CO_2 which equals $2{\cdot}94 \times 10^{-7}$ moles CO_2 per hour. This is an estimate of the supply of CO_2 from the root to the photosynthesising shoot under these conditions. It will be an underestimate as the radioactivity present in the shoot has not been considered.

The total leaf area of this plant is 23·1 cm^2 and this would indicate a whole plant requirement of between 85·9 - 182·0 moles $\times 10^{-7}$ CO_2 per hour. The supply from the roots ($2{\cdot}94 \times 10^{-7}$ CO_2 per hour) would equal 3·42 to 1·62 per cent of total requirement. The distribution of this supply from the root, between the shoot and the solution surrounding it, indicates that only 1·88% of this was retained in the leaves under these conditions.

Therefore, the supply of carbon dioxide from the roots, through the shoot, to the leaves is insignificant and it is unlikely that ^{14}C incorporation experiments, with detached leaves, will underestimate the rate of ^{14}C uptake by more than 2%.

CHAPTER THREE

THE EXOGENOUS INORGANIC CARBON ENVIRONMENT

3.1 Introduction

Aquatic and terrestrial environments differ with respect to the forms of inorganic carbon found in them. In air only carbon dioxide gas need be considered but in water carbon dioxide dissolves and dissociates into several forms of inorganic carbon, all of which may be potential sources or reservoirs of exogenous inorganic carbon for photosynthesis.

This chapter investigates the manner in which the availability of inorganic carbon to the surface of a broad-leaved pondweed can be affected by the chemistry of the carbonic acid system, both in the natural environment and in experimental enclosures.

3.2 The Carbonic Acid System of Natural Waters

Natural fresh waters, as aqueous solutions of electrolytes can be regarded as a solution of the corresponding free acids and bases (Ricci, 1952). The major inorganic solutes of natural waters are the carbonates, bicarbonates, sulphates and chlorides of calcium, magnesium, sodium and potassium, together with silica (Kemp, 1971 a). Thus a natural water may be regarded as containing carbonic, sulphuric, and hydrochloric acids together with the hydroxides of calcium, magnesium, sodium and potassium, as well as non-ionic silica (Ricci, 1952). The carbonic acid system contains the possible substrates for photosynthesis by freshwater macrophytes.

Carbonic acid (H_2CO_3) is dibasic forming carbonate and bicarbonate ions in water. When carbon dioxide is dissolved in water the following equilibria are established:

$$\underset{\text{(gas)}}{CO_2} \rightleftharpoons \underset{\text{(dissolved)}}{CO_2} \qquad (1)$$

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \qquad (2)$$

$$H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \qquad (3)$$

$$HCO_3 \rightleftharpoons CO_3^{2-} + H^+ \qquad (4)$$

$$CO_2 + OH^- \rightleftharpoons HCO_3^- \qquad (5)$$

$$H^+ + OH^- \rightleftharpoons H_2O \qquad (6)$$

Thus more CO_2 will dissolve in water than would be predicted by the gas solubility laws. Both dissociations are weak and therefore non-ionized molecules are always present in solution. The hydration reaction (2) is slow, with a half time of 15 sec at 15°C (Raven, 1970). Both the dissociations, however, are rapid compared to the hydration reaction. The ideal first dissociation constant of carbonic acid is:

$$\frac{[H^+][HCO_3^-]}{[CO_2][H_2CO_3]}$$

The ratio $\frac{[H_2CO_3]}{[CO_2]}$ at 25°C has been shown to be 0·0037 (Bell, 1959).

Therefore if the term $[H_2\ CO_3]$ is taken to include free carbon dioxide and non-ionised carbonic acid, the dissociation constant can be considered as:

$$K_1 = \frac{[H^+]\ [HCO_3^-]}{[H_2CO_3]} \qquad (7)$$

This has been called the apparent dissociation constant for the first ionization step of carbonic acid (Golterman, 1970). Various values of K_1 have been determined and are given in Table 3.1. The value used will be that given by Kemp (1971c) as this is a mean of several values from different sources.

The second dissociation constant K_2 is given by:

$$K_2 = \frac{[CO_3^{2-}]\ [H^+]}{[HCO_3^-]} \qquad (8)$$

Table 3.1

The values of K_1, the apparent first dissociation constant, and K_2, the second dissociation constant of carbonic acid.

K_1	$= 4.3 \times 10^{-7}$	Harned and Owen (1958)
K_1	$= 4.45 \times 10^{-7}$	Harned and Davies (1943)
K_1	$= 4.54 \times 10^{-7}$	MacInnes and Belcher (1933)
K_1	$= 4.32 \times 10^{-7}$	Shedlovsky and MacInnes (1935)
K_1	$= 4.43 \times 10^{-7}$	Kemp (1971)
K_2	$= 4.69 \times 10^{-11}$	Harned and Scholes (1941)

The equations 7 and 8 for the two dissociations are more useful when expressed in the following form (Henderson-Hasselbach):

$$pH = pK_1 + \log \frac{[HCO_3^-]}{[CO_3^{2-}]} \qquad (7a)$$

$$pH = pK_2 + \log \frac{[CO_3^{2-}]}{[HCO_3^-]} \qquad (8a)$$

From these equations the relationship of HCO_3^-, CO_3^{2-}, and H_2CO_3 to the pH of the water can be predicted. The dissociation constants K_1 and K_2 are temperature dependent and the changes in pK_1 and pK_2 that ensue are given in Table 3.2. It can, therefore, be seen that the ambient temperature will affect the ratios $[HCO_3^-] / [H_2CO_3]$ and $[CO_3^{2-}] / [HCO_3^-]$, although this is not significant. The relationship of free carbonic acid, bicarbonate and carbonate to total carbonic acid is as follows:

Total Carbonic acid = free carbon dioxide + bicarbonate + carbonate

$$(T.C.) = [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}]$$

The relationship of the three chemical species of carbonic acid, as a percentage of total carbonic acid, to pH, is given in Figure 3.3. Thus, when $[H_2CO_3] = [HCO_3^-]$ it can be seen from equation 7a that pH will equal pK_1, i.e. 6·35 at 25°C. Similarly when $[HCO_3^-] = [CO_3^{2-}]$ from equation 8a pH will equal pK_2, i.e. 10·33 at 25°C. It is also apparent that as the pH of

Table 3.2

The effect of ambient temperature on the first (apparent) and second dissociation constants, K_1 and K_2, of the ionisation of carbonic acid in water. (Harned & Owen 1958)

Temperature	pK_1	pK_2
0	6.58	10.63
5	6.52	10.56
10	6.46	10.49
15	6.42	10.43
20	6.38	10.38
25	6.35	10.33

$$pK_1 = -\log K_1$$

$$pK_2 = -\log K_2$$

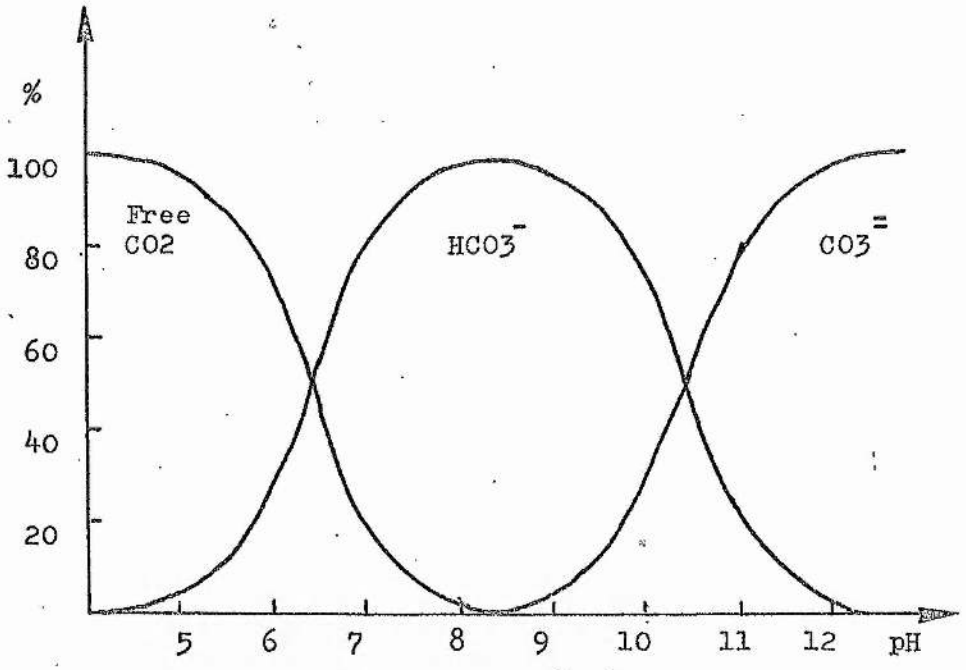


Figure 3.3

The relationship of the three chemical species of carbon in water with pH. After Schmitt (1955). Free carbon dioxide, bicarbonate and carbonate are expressed as a percentage of total "carbon dioxide".

water increases the proportion of free carbonic acid will decrease and approach zero at approximately pH 8·6. Therefore, in the range of pH found commonly in natural waters, bicarbonate ions will predominate.

However, this analysis of the carbonic acid system is for an ideal situation and correction for non-ideality should be made when analysing a fresh-water system in more detail. For instance, as the pH increases and the value of free carbon dioxide approaches zero this approximation becomes inaccurate. Thus, the system adopted by Kemp (1971) for non-ideality by introducing the appropriate activity coefficients, i.e.

$$K_1 \longrightarrow \frac{K_1}{Y2}$$

$$K_2 \longrightarrow \frac{K_2}{Y4}$$

can be used. The values of Y to be used can be determined from the Total dissolved solids (T.D.S.). Table 3.4 shows the relationship between Y and T.D.S. (Kemp, 1971). Then rearranging and substituting equations 5, 6, and 7 gives:

$$[H_2CO_3] = TC \quad \frac{[H]^2 + K_1[H] + K_1K_2}{[H]^2}$$

correcting for non-ideality and eliminating TC after Kemp (1971) gives:

$$[H_2CO_3] = \frac{[H]^2 \; Y^6}{K_1[H]Y^4 + 2K_1K_2} \; . \; T.A.$$

Table 3.4

The values of the univalent activity coefficient Y as a function of Total Dissolved Solids (mg/l) (Kemp, 1971)

T.D.S.	Y
150	0.940
160	0.938
170	0.936
180	0.934
190	0.932
200	0.930
300	0.922
400	0.904
500	0.894
600	0.885

T.D.S. (mg/l) = Conductivity (μ ohms^{-1}cm^{-1}) x 0.67.

Thus, to know the $[H_2CO_3]$ value it is necessary to know the total alkalinity (T.A.), the pH, and the conductivity or total dissolved solids. As this equation is cumbersome, a computer programme was written to produce tables of its solution for any combination of the three input variables (Table 3.5). A set of these tables produced on the computer is given in appendix 1. By changing the table parameters for alkalinity and pH a set of tables may be produced for waters of different alkalinity or pH ranges. To use the tables it is first necessary to select the table with the corresponding alkalinity (E = T.A. in m eq l^{-1}) to that of the water under study. The choice of the column, on the table, is made by calculating the value of Y, the univalent activity coefficient, from the total dissolved solids of the water. The column then gives the value of free carbon dioxide (moles x 10^{-3}) for the value of pH as indicated in the extreme left hand column.

Using these tables, the effect of total alkalinity on the relationship between pH and free carbon dioxide concentration was examined. Figure 3.6 shows this relationship for total alkalinities of 3·6 and 3·15. It can be seen that total alkalinity has a small effect on free carbon dioxide concentration compared to the effect of pH. Similarly, the effect of total dissolved solids on the relationship between free carbon dioxide and pH is given in Figure 3.7. This shows that the free

Table 3.5

The computer program written in Fortran 1V to solve the equation for concentration of free carbon dioxide after Kemp (1971).

$$f = \frac{H^2 . Y^6}{K_1 . H . Y^4 + 2 . K_1 . K_2} \cdot e$$

```
1. REAL K1 / 4.43E-7 /,K2/4.69E-11 /, E(50),Y(50),F(50)
2. READ(5,30) PHMIN,PHMAX,NPH
3. READ(5,10) NE,(E(I),I=1,NE)
4. READ(5,10) NY,(Y(I),I=1,NE)
5. PHINC=(PHMAX-PHMIN)/(NPH-1)
6. PHMIN1=PHMIN-PHINC
7. DO 9 J=1,NE
8. WRITE(6,20) E(J),(Y(I),I=1,NY)
9. WRITE(6,50)
10. DO 9 JPH=1,NPH
11. PH=PHMIN1+JPH*PHINC
12. H=10.0**(-PH)
13. DO 8 JY =1,NY
14. F(JY)=(H*H*Y(JY)**6*E(J))/(K1*H*Y(JY)**4+2.0*K1*K2)
15. WRITE(6,40) PH,(F(JY),JY=1,NY)
16. STOP
17. FORMAT(I2,7(7(1X, E10.4)/))
18. FORMAT('1 TABLE FOR  E= ',E12.5/'+ ',26('_')/'0 Y= ',50(1X,E8.3))
19. FORMAT(2(E10.4,1X),I3)
20. FORMAT(1X,F4.2,50(1X,E8.3))
21. FORMAT(1X)
22. END
```

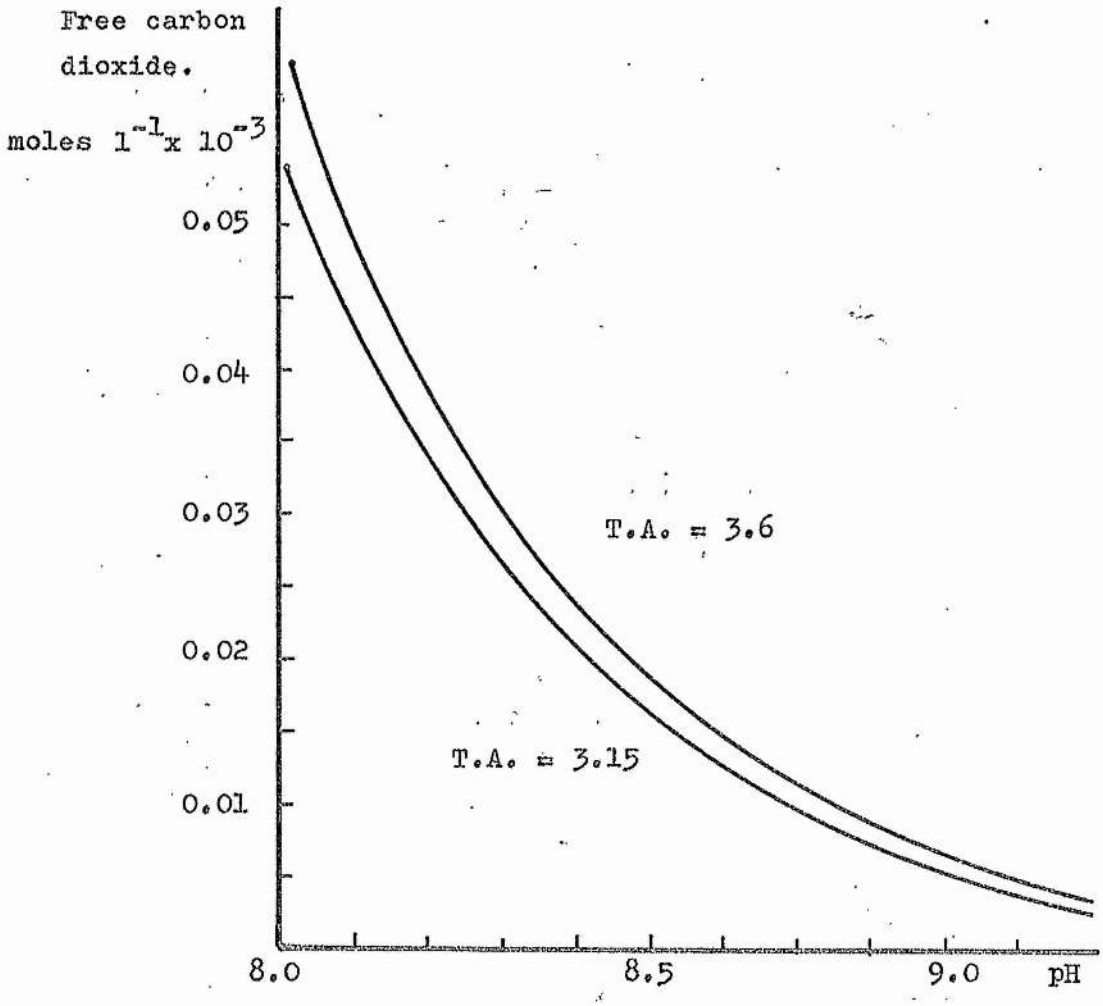


Figure 3.6

The effect of Total Alkalinity on the relationship between the pH and the free carbon dioxide for a loch water of T.D.S. = 600 mg l^{-1} . Drawn from the tables in appendix I. The two lines represent two values of alkalinity of 3.15 and 3.6 m eq.l^{-1}.

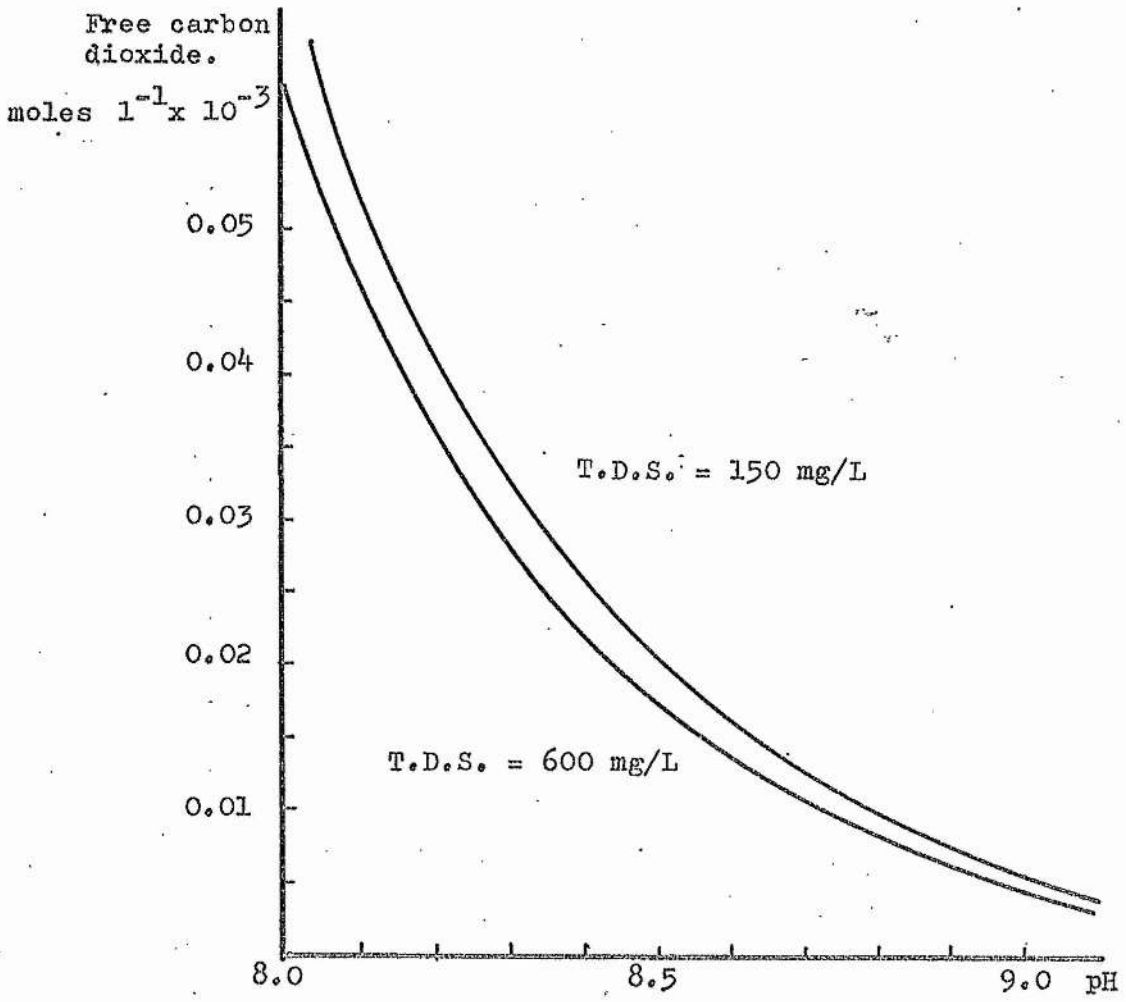


Figure 3.7

The effect of total dissolved solids on the relationship between pH and free carbon dioxide for a loch water of alkalinity 3.45 m eq.l^{-1}. Drawn from the tables in appendix I. The two lines represent the extreme values of T.D.S. (150 and 600 mg l^{-1}) of natural freshwaters.

carbon dioxide is affected to a small extent by total dissolved solids compared to pH.

The concentration of free carbon dioxide, in a closed system, is therefore significantly more dependant on the ambient pH than on either the total dissolved solids or the total alkalinity of the loch water under study. Thus, in any estimation of the free carbon dioxide content of a loch water the accuracy of the pH determination is of prime importance.

The significance of pH on the concentration of free carbon dioxide is demonstrated by a comparison of its concentration with that of air. The average terrestrial carbon dioxide concentration is about 300 v.p.m. which is $1{\cdot}2 \times 10^{-5}$ moles l^{-1}. The free carbon dioxide in a calcareous loch of $3{\cdot}2 \times 10^{-3}$ M T.C. (Appendix I) ranges from 6×10^{-4}M at pH 8·0 to $5{\cdot}4 \times 10^{-5}$M at pH 9·0.

Analysis of the Carbonic acid environment in Freshwaters from Lochs in the area studied

Introduction The analysis of the carbonic acid system of a loch water sample requires the measurement of the pH, the conductivity, and the titration of the sample to determine the total alkalinity.

There are two different approaches to measuring the pH of a loch water. A field instrument can be used directly or a sample can be collected and brought to a laboratory for measurement with a more accurate meter and electrode. There is the possibility of changes

occurring, after collection and before analysis, if the carbon dioxide gas in the sample is substantially above or below the air equilibrium value. Errors in the field measurement may also be caused by the inferior equipment used.

Therefore, to eliminate these two main sources of error in pH measurement, samples were collected and taken quickly to a field laboratory for determination on a good quality pH meter. For particular studies in the changes in pH of a given loch water the same pH meter was set up at the loch side using a portable petrol-powered generator for a power supply.

Method The samples of loch water were collected in 500 ml screw-cap jars under water, sealed to exclude air bubbles and to prevent gaseous exchange with the atmosphere. These samples were then taken to the field laboratory and analysed immediately. Samples could be collected from any location on the loch surface or up to any depth colonised by macrophytes by means of aqualung diving.

An aliquot of this sample was taken and titrated against 0·02M HC1 standard acid. This acid was freshly made up using distilled water that had been boiled for at least an hour and cooled with a soda lime tube to prevent any reabsorption of carbon dioxide. The apparatus used to perform the titration is shown in Figure 3.8, the two soda-lime tubes allowing the

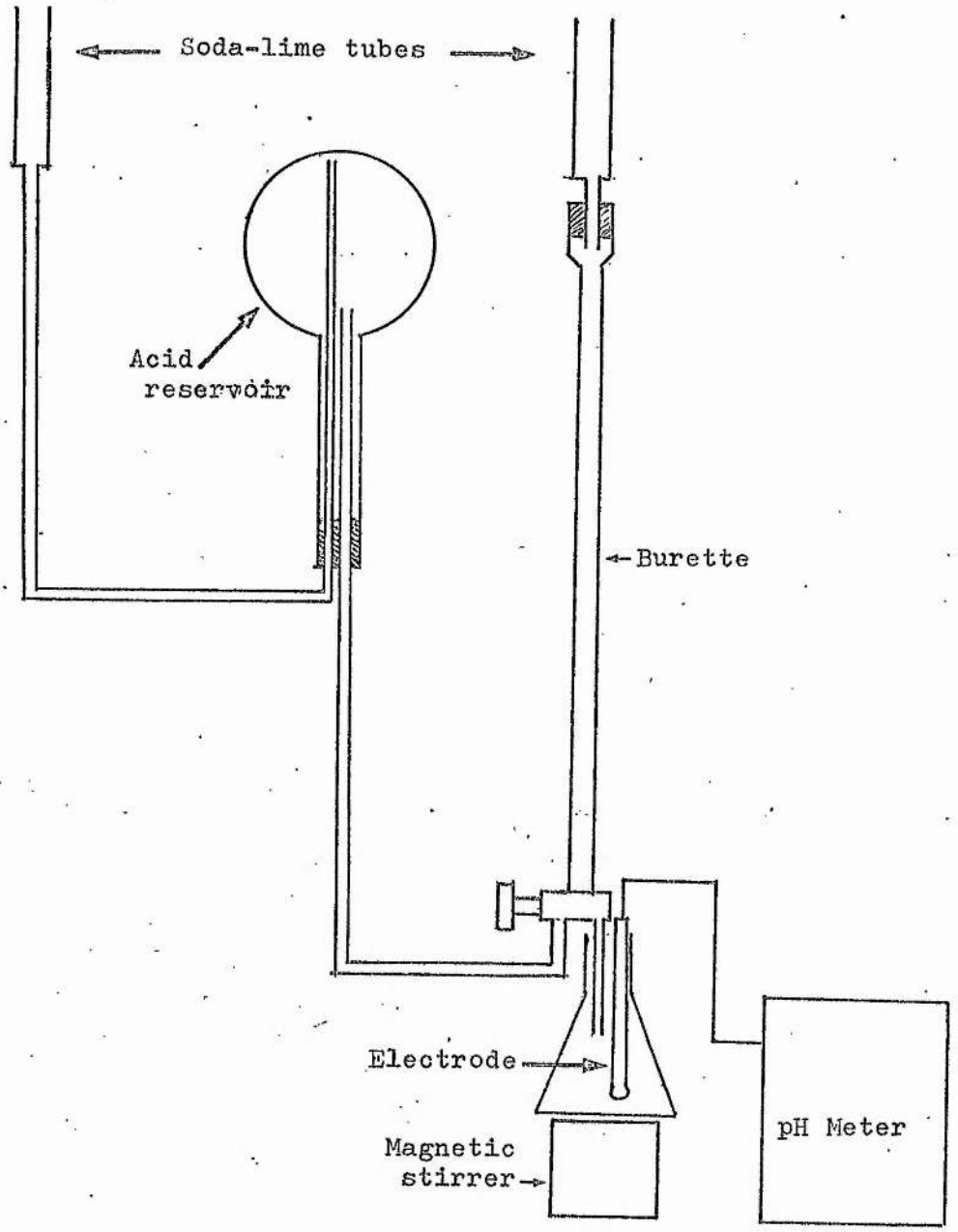


Figure 3.8

The apparatus set up in the field laboratory for titration of loch water samples. The burette is refilled between titrations directly from the acid reservoir, the soda-lime tubes preventing entry of carbon dioxide to the system. The magnetic stirrer kept the contents of the flask mixed during the titration.

burette to be filled from the standard HC1 reservoir without allowing any absorption of carbon dioxide from the atmosphere. Thus, when the apparatus was set up many samples could be analysed rapidly and in succession.

The magnetic follower in the titration flask ensured that the acid added was mixed rapidly and that the pH electrode read correctly. The individual titrations were carried out as quickly as possible to prevent exchange between atmospheric carbon dioxide and the contents of the flask becoming significant. The samples were first taken to an end point of pH 8·3, if already above this, and then titrated to an approximate end point of about pH 5·0. An approximate value of T.A. was then calculated and knowing the initial pH and conductivity range of the sample the approximate total carbonic acid was calculated using Tables II.1 and II.2 in Appendix II after Golterman (1969). The true end point pH is then read off Table II.3 and the sample titration continued to this. The volume of standard acid required to get to this end point gives the T.A. The total carbonic acid is also calculated.

Results Samples were taken from four limestone lochs (L. Borralie, L. Caladail, L. Croispol and L. Lanlish) and from one peat loch (L. Meadie) in the area studied in Durness. Samples were collected at various locations and depths corresponding to the range of vegetation present. One such set of results for these lochs

is given in Table 3.9. The values of pH, total alkalinity and total carbon dioxide of the four limestone lochs are similar and in contrast to the non limestone loch. The pH and T.A. values of the limestone lochs show that although these waters have a high total carbon dioxide, there will only be a small amount of this present as free carbon dioxide. This will be biologically significant ranging from several times the atmospheric concentration around pH 8 to several times less at ph 9.

Table 3.9

Analysis of water samples from selected lochs in the Durness area in May 1973. The total alkalinity and the total carbon dioxide are given in units of $m.eq.l^{-1}$.

	Borralie	Caladail	Croispol	Lanlish	Meadie
pH	8.66	8.72	8.37	8.34	7.14
TA.	2.53	3.02	3.44	2.56	0.012
TC.	2.504	2.986	3.44	2.56	0.0142

3.3 Dissolved Carbon Dioxide

All dissolved gases are held in solution by their respective partial pressures in the gas phase associated with the liquid phase (Henry's law). Therefore, when carbon dioxide partitions itself between the gas and liquid phases, by diffusion, the amount in solution will be in equilibrium with the partial pressure (pCO_2) in the gas phase. Thus, the solubility coefficient will be defined as

$$\text{Dissolved } CO_2 \text{ (moles } l^{-1}) = pCO_2 \cdot \alpha$$

However it will now be important to make the distinction between dissolved carbon dioxide gas and hydrated carbon dioxide as in equation 2. The gas solubility law will apply only to the dissolved gas.

Although there are seasonal changes in the concentration of carbon dioxide in the atmosphere (Bischoff, 1960), for the purpose of the present study it is reasonable to consider the atmospheric partial pressure of carbon dioxide above a loch water to be constant. The solubility will be temperature dependent and the variation of the solubility coefficient with temperature is given in Figure 3.10.

Photosynthesis and respiration by aquatic plants will cause fluctuations in the dissolved carbon dioxide and total carbonic acid of the loch water. The dissolved carbon dioxide changes will cause a displacement in reactions (1) and (6) to form a new series of

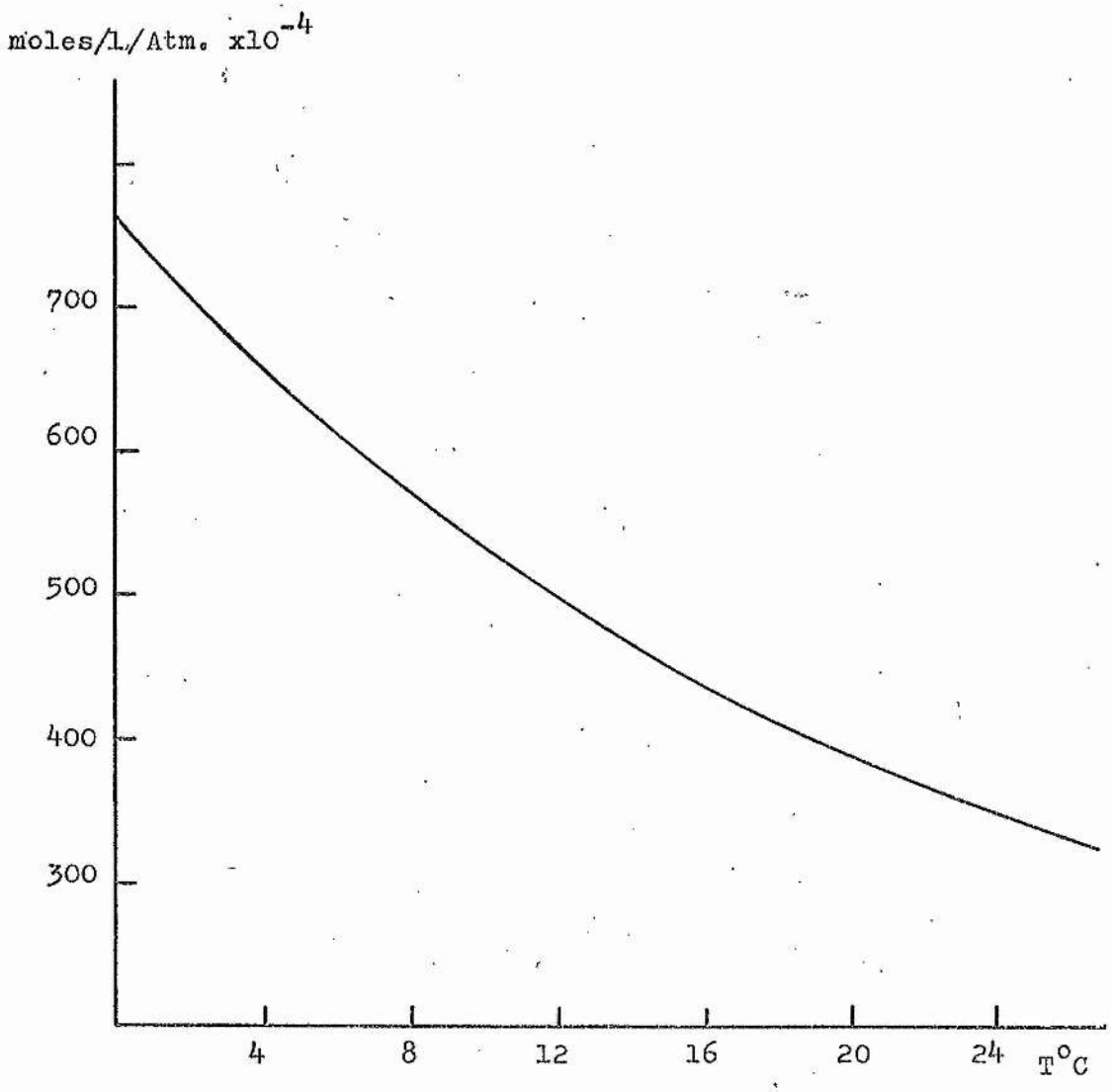


Figure 3.10

The variation of the solubility coeficient of carbon dioxide in water with temperature. (Drawn from data given in Riley and Chester, 1972.) The solubility coeficient is given from:

$$\frac{\text{Conc. dissolved } CO_2}{\text{Partial pressure } CO_2} \qquad \frac{(\text{moles/l})}{(\text{Atm})}$$

equilibria. The magnitude of the shift of equilibria will be related to the 'pCO_2' buffering capacity of a water type for changes in total carbonic acid (Kanwisher, 1963). The net effect of this will be that in a water of given alkalinity the change in the dissolved carbon dioxide, that will occur for a given change in total carbonic acid, will be less than that of distilled water. This difference in change of dissolved carbon dioxide will be related to the alkalinity of the loch water concerned. The difference in 'pCO_2' buffering capacity for waters of different alkalinity has been experimentally determined (Kanwisher 1960). Figure 3.11 shows this difference in 'pCO_2' buffering capacity for fresh distilled water and sea water, where the same change in total carbonic acid produces a much larger change of dissolved carbon dioxide in distilled water than sea water.

The higher the alkalinity the greater the 'pCO_2' buffering capacity and hence the dissolved carbon dioxide increase or decrease will be partially absorbed, that is reduced to a smaller change than would occur in distilled water. The remaining difference between the dissolved carbon dioxide and 'pCO_2' of the atmosphere will be equilibrated by net diffusion through the gas-liquid phase interface. It can be shown that the exchange between gaseous carbon dioxide and water may be slow compared to other gases such as oxygen. Thus, a dissolved oxygen deficit is more quickly replaced by

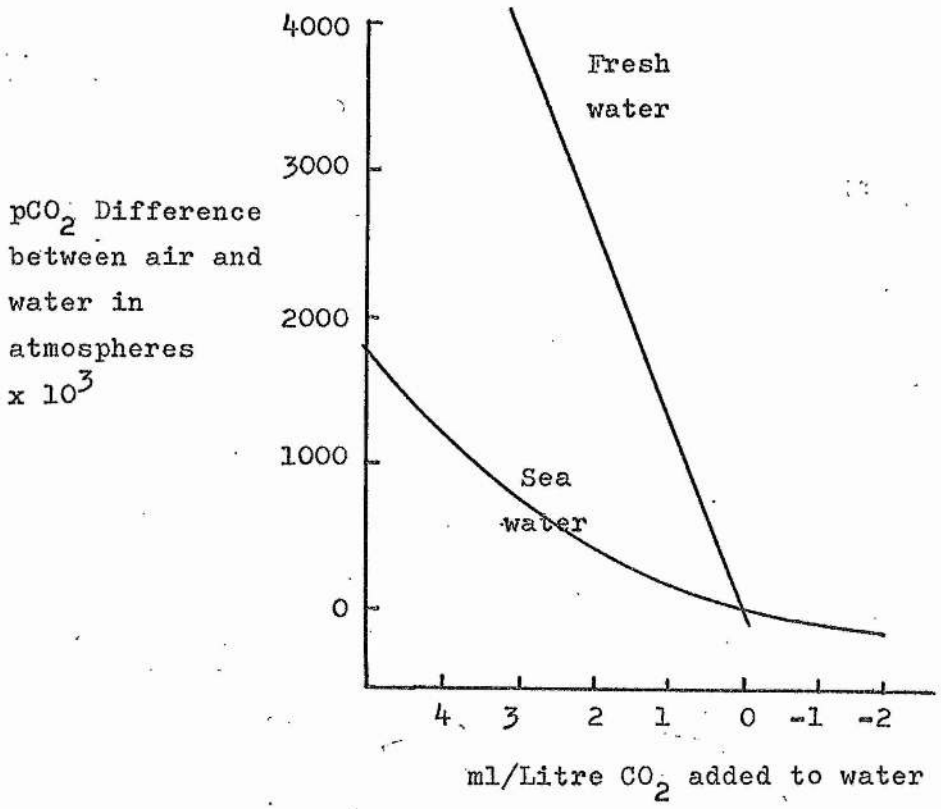


Figure 3.11

The experimentally determined partial pressure variation of carbon dioxide with changes in total carbon dioxide in sea and fresh water. From Kanwisher (1960)

diffusion from the gas phase than a carbon dioxide deficit. This is mainly due to the vastly different partial pressures of oxygen and carbon dioxide in the atmosphere rather than a significant difference in the diffusion of oxygen and carbon dioxide in water. The wind velocity and associated turbulence of the water surface have been shown to increase the movement of carbon dioxide across the air-water boundary (Kanwisher, 1962, 1963).

Thus, as the return to equilibrium of carbon dioxide between the gas and liquid phase will be diffusion limited in the liquid phase, it will be slow. Differences between dissolved and gaseous carbon dioxide caused by biological activity, in water, may persist for several months (Teal and Kanwisher, 1966; Talling, 1976).

The following investigations were undertaken to measure the extent of pH changes in water bodies due to a net removal of carbon dioxide by photosynthetis activity of macrophytes.

<u>To Demonstrate that an Aquatic Macrophyte will remove Dissolved carbon dioxide from Pond Water faster than the diffusive supply from the Atmosphere</u>

<u>Method</u> A glass tank (18" x 12" x 24") containing growing <u>P. perfoliatus</u> plants collected from L. Croispol and rooted in soil, was placed in the laboratory window where it would receive sunlight during the day. A pH electrode was placed so that its tip was two inches

below the surface of the water, and connected to a Pye model 290 pH meter. The temperature correction circuit was set to automatic and the sensory element positioned in the tank next to the pH electrode as in Figure 3.12. A Pye model SP22 chart recorder was connected to the output of the pH meter and the pen adjusted so that the trace on the paper corresponded to the reading on the meter. The chart paper speed was set at the slowest setting of 50 min. per cm. and the system left to record the pH for a period of seven days.

Results A copy of the trace obtained is shown in Figure 3.13. The diurnal variatipn of the pH in the tank water is apparent, with the minimum pH occurring at about 0700 hrs. and the maximum pH occurring at about 1900 hrs. Table 3.14 shows the maximum and minimum pH measured for each period of 24 hours and the pH range in that period. The average diurnal pH change is 0·62 pH and the variance in diurnal change is probably related to the quantity of sunlight incident in each 24 hr. period. The pH maximum occurred at the end of day, after the period of photosynthesis and the pH minimum occur at the end of the night, after the period of respiration.

There is also an overall trend for the pH in the tank to increase during the period of measurement. Thus, the pH minimum and pH maximum values increase by 0·44 pH and 0·48 pH units respectively.

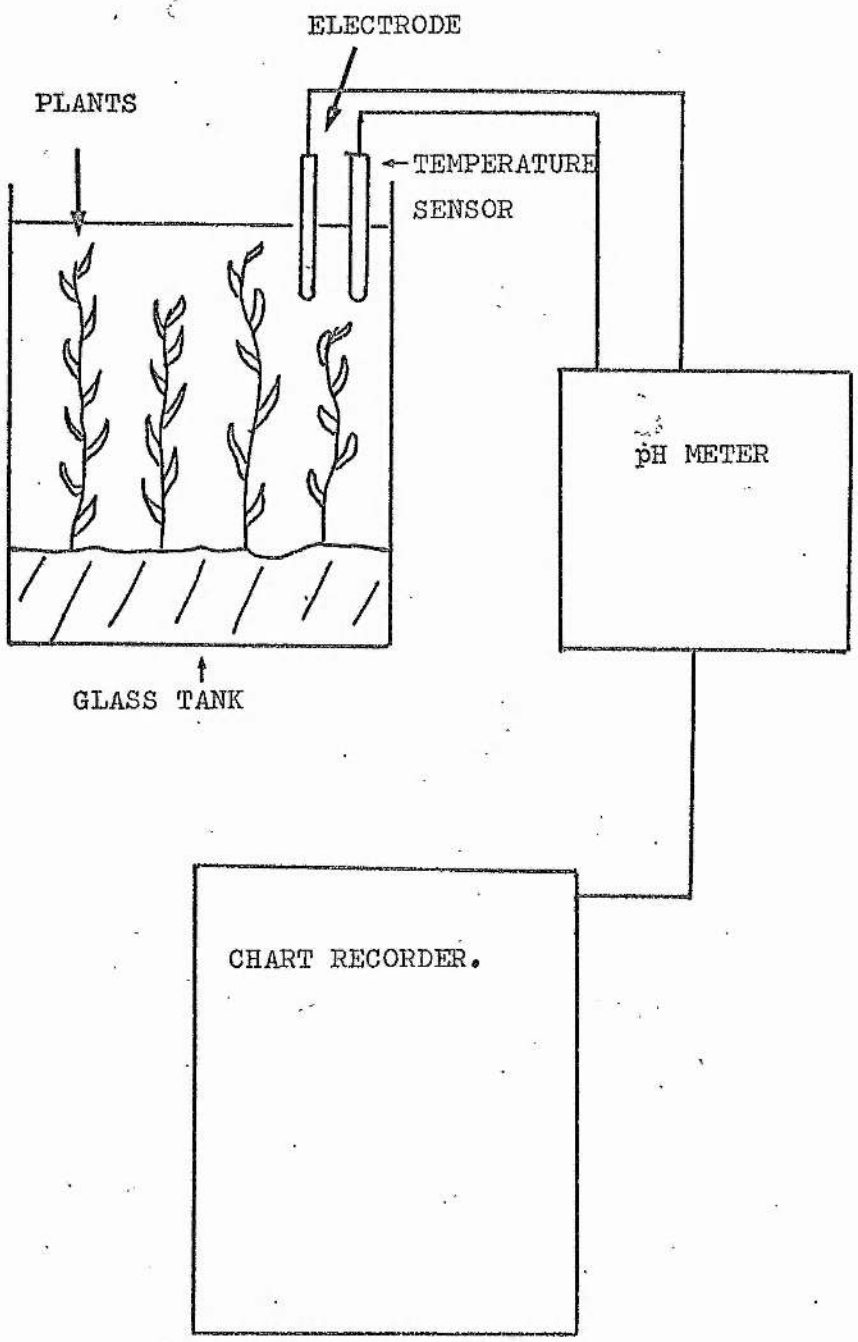


Figure 3.12

The laboratory experiment to observe the change in pH of the water in a glass tank of planted P. perfoliatus. The tank was placed in the window of the laboratory where it would receive direct sunlight

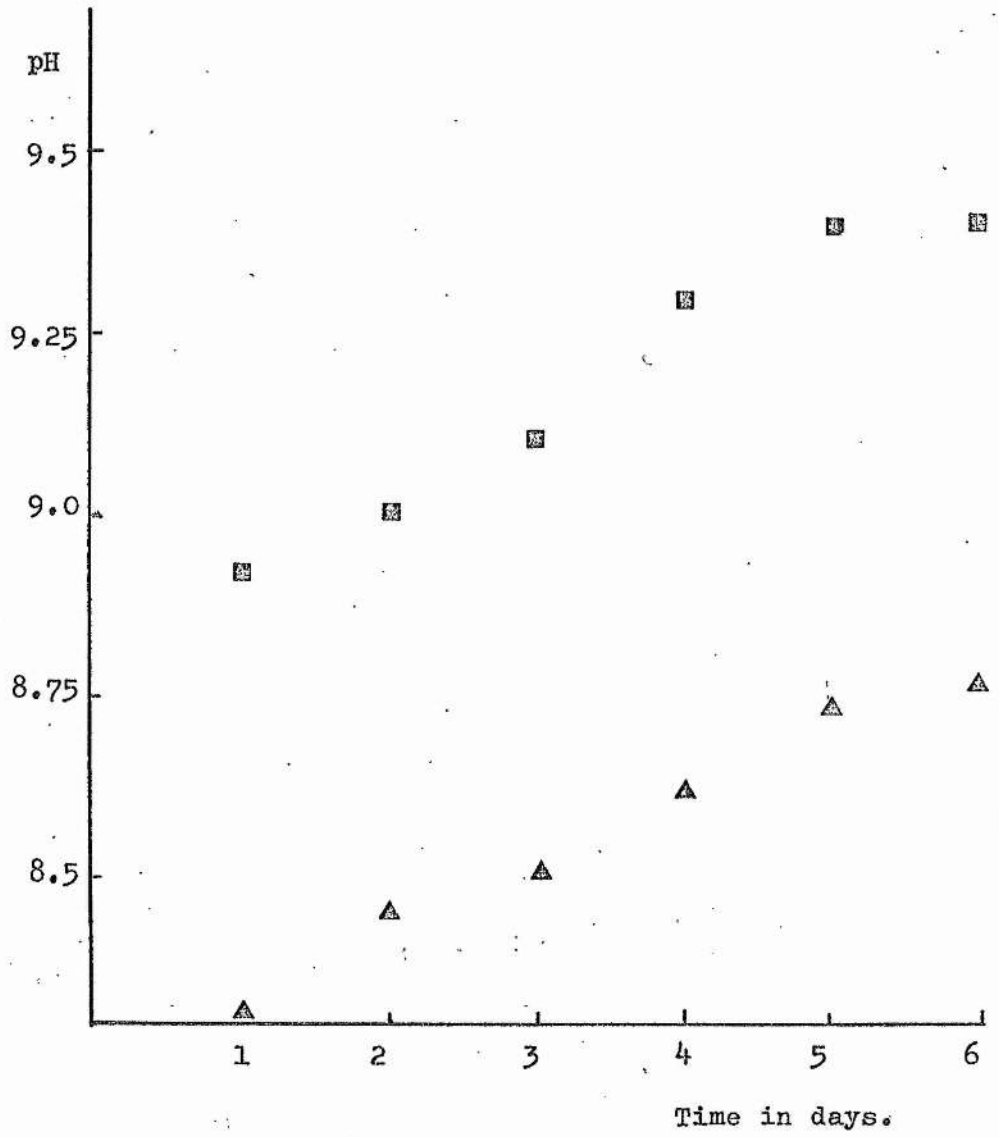


Figure 3.13

The drift in diurnal maximum and minimum pH occuring in a glass tank in the laboratory over a period of six days.

Maximum pH = ■ , Minimum pH = ▲ .

Table 3.14

The maximum and minimum pH values found in each succesive period of 24 hours and the ranges found for the same periods. The time of day at which these tended to occur and the overall drift in pH over the period of observation are also given.

Day.	7am. pH min.	7pm. pH max.	pH change.
1	8.32	8.92	0.6
2	8.45	9.00	0.55
3	8.50	9.11	0.61
4	8.62	9.29	0.67
5	8.73	9.39	0.66
6	8.76	9.40	0.64
pH drift.	0.44.	0.48.	

Discussion The density of plants in the tank was similar to that found in their natural habitat. However, it is immediately apparent that the production and consumption of carbon dioxide by respiration and photosynthesis of aquatic macrophytes can affect the pH of the surrounding water significantly. The magnitude of the change will be related to the ratio of the amount of biological material to the volume of the water affected. The ratio of the water surface area in the tank to its volume will affect the rate of exchange of atmospheric carbon dioxide with the tank water as a whole. In fact, it would be reasonable to conclude that two factors will make the pH change observed in the experiment smaller than might occur in natural situations. Firstly, as the experiment was conducted in the laboratory, the pCO_2 in the atmosphere would be higher as a result of human respiration and the entry of carbon dioxide would be aided. Secondly, the tank only represents the situation near the surface of a loch in that it is shallow and more able to equilibrate quickly with atmospheric carbon dioxide as the diffusion path will be smaller.

But other factors will reduce the exchange of carbon dioxide compared to the natural situation. Firstly, relatively speaking, the air in the laboratory will have an insignificant velocity compared to that above a loch water surface. Secondly, the movement of the water by currents and waves will be very small

compared to that occurring naturally. These two factors will together reduce the exchange of carbon dioxide with the body of the water in the tank and lead to greater pH changes than might occur naturally.

It is significant to notice also that the general drift in average pH during the course of the experiment (Table 3.14), showed that there was net photosynthesis by these plants and its effect on the surrounding water is to remove carbon dioxide faster than it can be replaced from the atmosphere.

It is, therefore, reasonable to expect that the trends shown by this experiment will occur in the loch environment but the extent to which they will occur will depend upon factors such as wind, current velocity depth, plant density, photosynthesis and respiration rates.

To Measure the Diurnal pH Change of Two Loch Waters in a Weed Bed

The glass tank experiment in the laboratory showed that the change in pH of pond water around photosynthesising aquatic macrophytes could be significant. However, there were too many variables affecting the magnitude of the change to allow anything but a qualitative extrapolation to the natural situation. Therefore, this experiment was set up to ascertain the magnitude of diurnal pH changes to be expected.

Method A piece of rubber tubing was clamped in a retort stand so that it pointed upwards. This stand

was then placed by aqualung diving at the sampling point, in the weed under study, with the apperture being about 20 cm. from the bottom of the loch. The tubing was then uncoiled along the loch bottom until the shore was reached. Here it was connected to a peristaltic pump and then to a constant-head chamber containing a pH electrode (Figure 3.15). The pump and the pH meter were operated and powered by a portable petrol-driven generator. When the pump was switched on, water from the sampling point flowed past the electrode in the constant head chamber and the pH could be read directly on the pH meter. The length of time required for the pump to clear the 'dead' space of fluid in the tube was determined by experiment and it was run for at least twice this time before any pH readings were taken. The thermometer fitted to the constant head chamber was used to adjust the temperature compensator on the pH meter when a reading was taken. It also provided a record of the temperature of the water at the sqmpling point. The pump, pH meter and generator were switched off after each reading had been taken and switched on about one half hour before the next reading to allow the meter to stabilise. A series of readings was taken over a period of at least 24 hrs. for each sampling site.

The first sampling site was at a depth of 5 feet in a bed of P. perfoliatus, in Loch Croispol. The period of measurement was between 8.8.73 and 9.8.73. The second

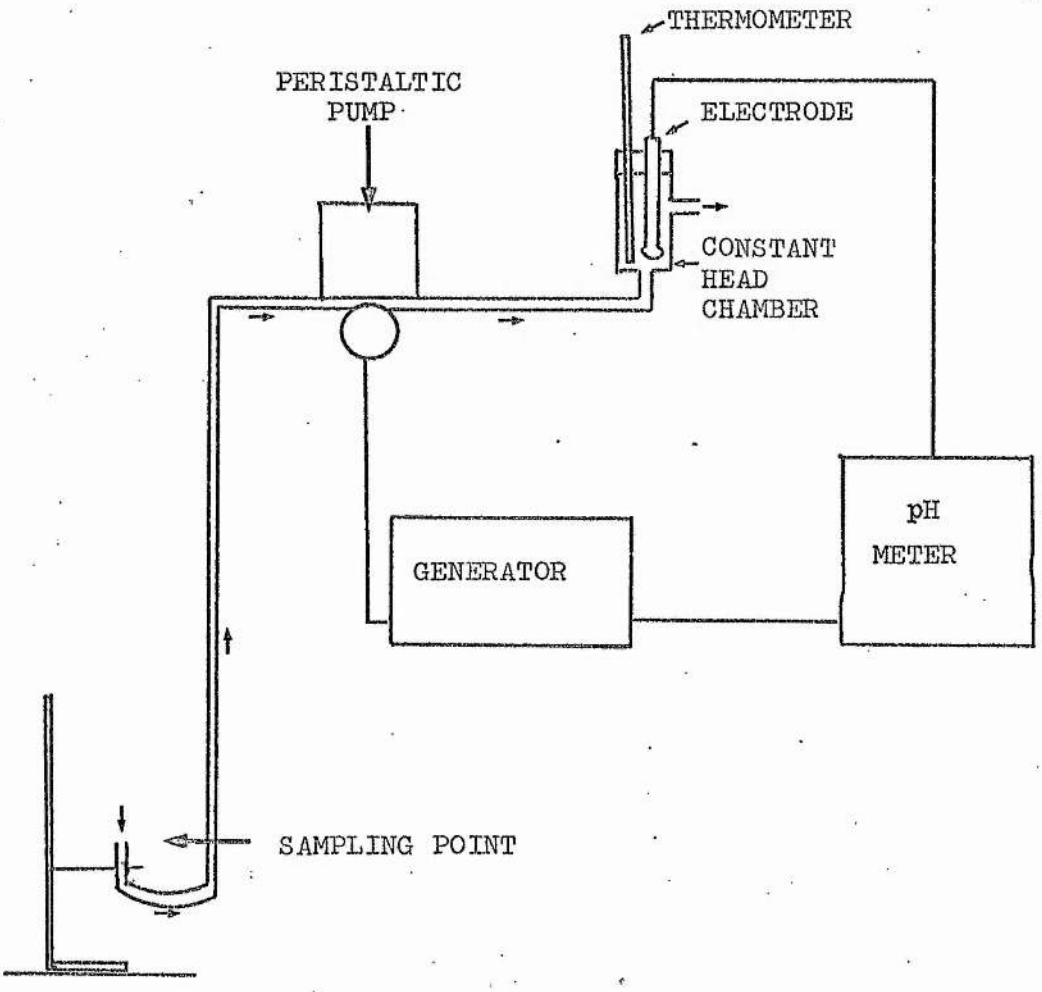


Figure 3.15

The apparatus set up at the loch side to measure the diurnal pH change of the water in a weed bed. The generator is petrol driven and provides the power for the pump and the pH meter. Readings of the pH meter and thermometer were taken manually. The direction of flow of the water is given by the arrows.

site was at a depth of 10 feet in Loch Borralie in a bed of Chara sp. This was measured on the 10.8.73 to 11.8.73. The weather during these periods was bright and sunny with very little cloud. There was not much wind or water turbulence.

Results The pH values for the water sampled from each loch are plotted in Figures 3.16 and 3.17. The diurnal pH variation can be seen to occur in both lochs. The difference between maximum and minimum pH is about 0·2 units. The maximum pH in both lochs has occurred shortly after 1500 hours and the pH minimum at about 0300 hours.

The insert in each figure shows the temperature variation that is occurring on a diurnal basis. This is to be expected from the difference in solar irradiance during a 24 hr. period. The difference in temperature of the two sampling points in the two lochs is probably due to their different depths.

Discussion A similar pattern of diurnal variation can be seen in Figures 3.16 and 3.17 to that formed in the laboratory experiment with the glass tank. However, the difference between pH minimum and maximum (0·2 pH units) is about half that in the laboratory. This field range was measured under conditions of minimum wind, and therefore water movement, and maximum solar irradiance. Under these conditions pH changes are likely to be higher than those found on days when the water mixing is greater or the solar irradiance is lower.

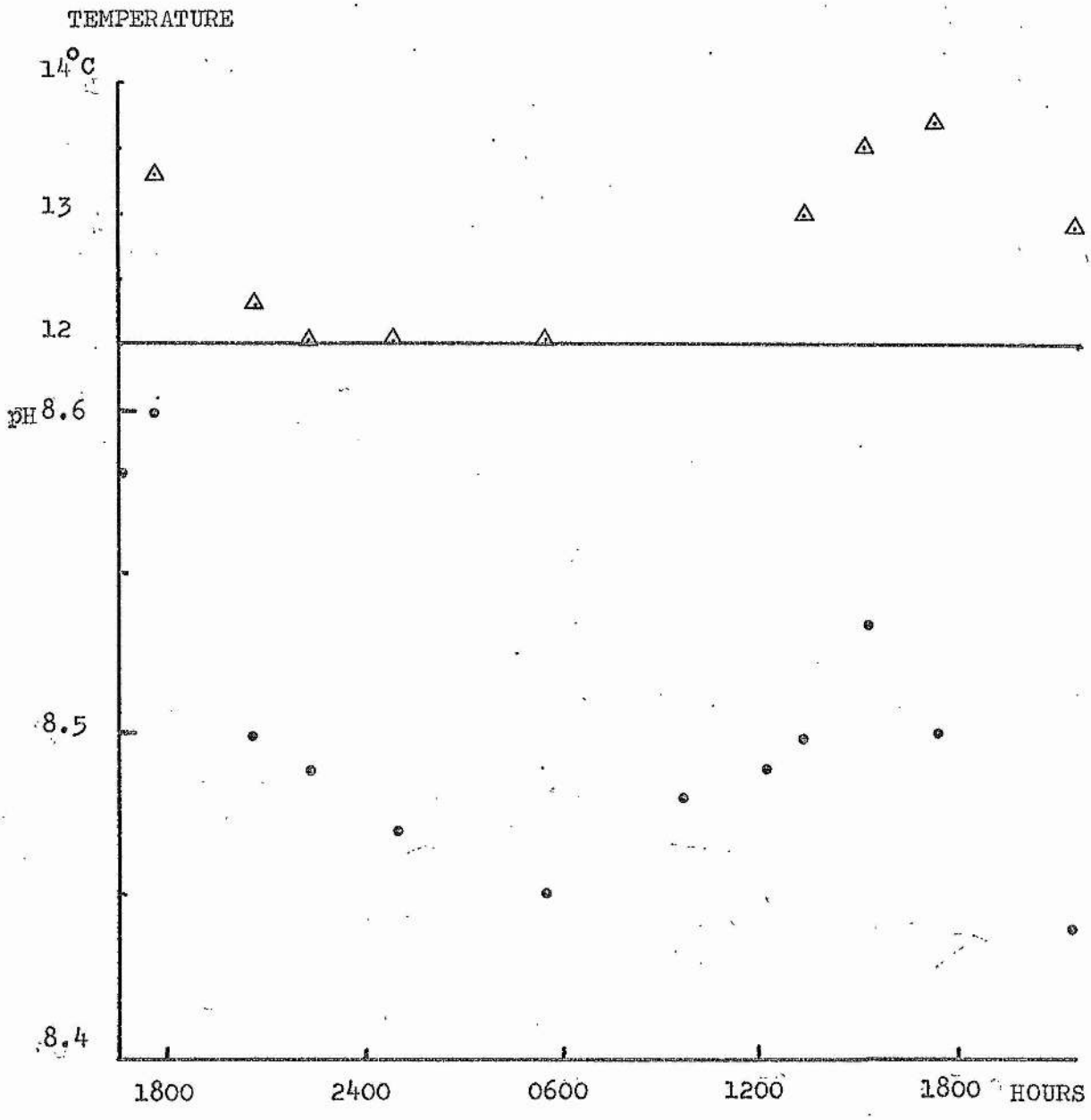


Figure 3.16

The diurnal variation in pH of water from a depth of 10 ft in Loch Borralie measured between 10/8/73 and 11/8/73. The sampling point was in a bed of Chara sp.. The insert shows the corresponding variation of temperature during this period.

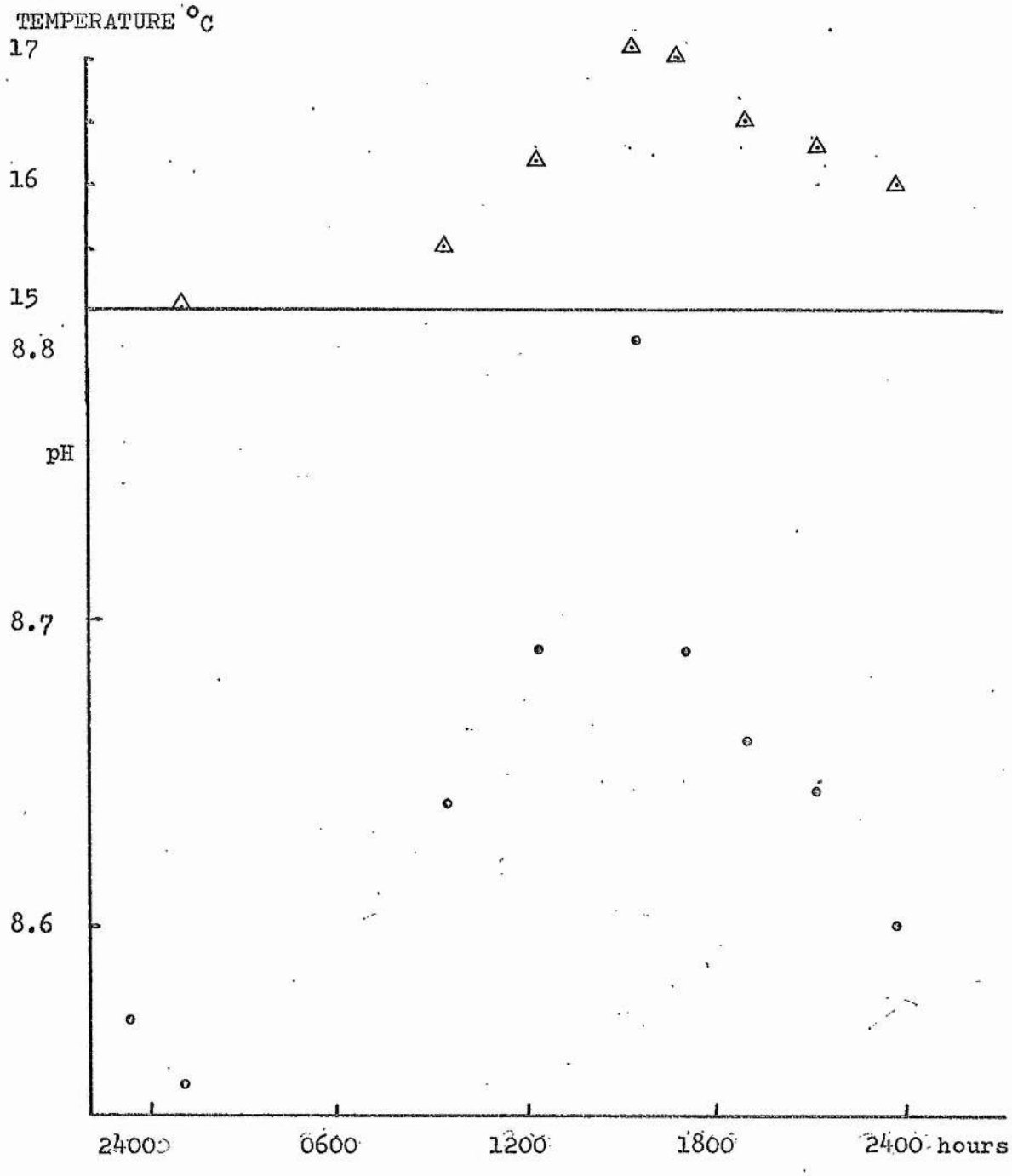


Figure 3.17

The diurnal variation of pH of water from a depth of 5 ft. in Loch Croispol measured between 8/8/73 and 9/8/73. The sampling point was in a bed of P. perfoliatus. The insert shows the corresponding variation of temperature during this period.

The alkalinity of Loch Croispol (3·3 meq l^{-1}) is higher than Loch Borralie (2·5 meq l^{-1}) and therefore Loch Croispol would be expected to have a greater pCO_2 buffering capacity and hence show smaller pH changes for the same change in total carbonic acid. That it shows a larger pH change is in agreement with the observation that the biomass of its P. perfoliatus beds is higher than the Cara beds in Loch Borralie and that the sampling depth in Croispol is about half.

Therefore, the diurnal pH changes in the loch environment (0·1-0·2 pH) are much smaller than occurred in a laboratory tank (0·55-0·67 pH). It was further decided to examine the range of pH changes occurring in experimental enclosures during a typical in situ photosynthesis experiment.

3.4 The pH Changes Observed During an *in situ* photosynthesis Experiment

Experimental The *in situ* experiment was carried out on single detached leaves of *P. pefoliatus* in Lake Garda, Italy. Leaves were collected at the site of incubation, and sealed in 25 cm^3 MaCartney bottles. The bottles to be used as dark enclosures were wrapped in aluminium foil and all the bottles were placed on a frame at the depth of 2·4 metres. The incubation period was for three hours around noon. Samples of the lake water were taken to the surface and the initial pH measured. Samples were also taken to the laboratory and the total alkalinity of the water determined by titration.

At the end of the incubation, when the bottles were brought to the surface for completion of the photosytnesis experiment, measurements of the final pH in each bottle were made.

Results The initial pH was 8·3 and the alkalinity of the lake water 2·2 meq l^{-1}. The change in pH, for each bottle, during the course of the experiment is given in Table 3.18 along with the treatment and area of the leaf in that bottle. The light enclosures show an increase of about 1 pH while the dark enclosures show an average decrease of 0·425 pH.

Discussion These pH changes, in light and dark enclosures, during a typical *in situ* photosynthesis experiment are markedly larger than those occurring naturally

Table 3.18

The pH changes observed during an "in situ" determination of the rates of photosynthesis of leaves of P. perfoliatus at a depth of 2.4 metres in Lake Garda, Italy. The initial pH and alkalinity were 8.3 and 2.2 m.eq./l respectively. The area of each leaf, in cm^2 is also given and the duration of the experiment was 3 hours.

Treatment	Change in pH	Leaf area in cm^3
Light	+ 0.95	6.9
Light	+ 1.1	7.1
Dark	- 0.5	5.4
Dark	- 0.35	5.5

in a weed bed or in a laboratory tank. They are the result of the addition or removal of carbon dioxide to or from the carbonic acid system in a small volume and are of such a magnitude that the concentration of free carbon dioxide can become several times smaller or larger. These field photosynthesis experiments are not, therefore, in situ with respect to availability of external inorganic carbon, as this can change significantly during the course of an incubation.

Therefore, a more critical examination of the factors affecting the magnitude of the pH changes, occurring in enclosures, was undertaken in the laboratory.

3.5 The Effect of Incubation Vessel Size and Amount of Plant Material per Vessel on the pH of the Incubating Fluid During the Measurement of Photosynthesis

Experimental Leaves of P. pefoliatus were cut from healthy shoots growing in an artificial pond in the greenhouse and each was sealed, with its own pondwater in glass bottles with all air bubbles excluded. The pH of the pondwater was measured and the bottles placed on the bottom of a constant temperature bath as in Figure 3.19. The lamps were switched on and the bottles incubated at $20^{o}C$ for 5 hours. At the end of the incubation period the pH of each bottle was measured and its leaf removed and placed in a previously dried aluminium foil cup to determine the oven dry weight. Ten replicate bottles were used and the experiment was repeated using different sets of bottles ranging in size from 25 cm^3 to 500 cm^3.

Results The pH change per mg tissue oven dry weight was calculated for each bottle and the averages obtained for each set of bottle sizes are given in Figure 3.20. The effect of tissue dry weight on the pH change in the 25 cm^3 bottles is shown in Figure 3.21.

Discussion The results show that the use of single detached leaves in small enclosures (25 cm^3 bottles), filled with the water in which they had been growing, for photosynthetic incubations produces pH increases which may be as high as 1·5 pH (Figure 3.21). Increasing the size of the vessel reduces this change and in

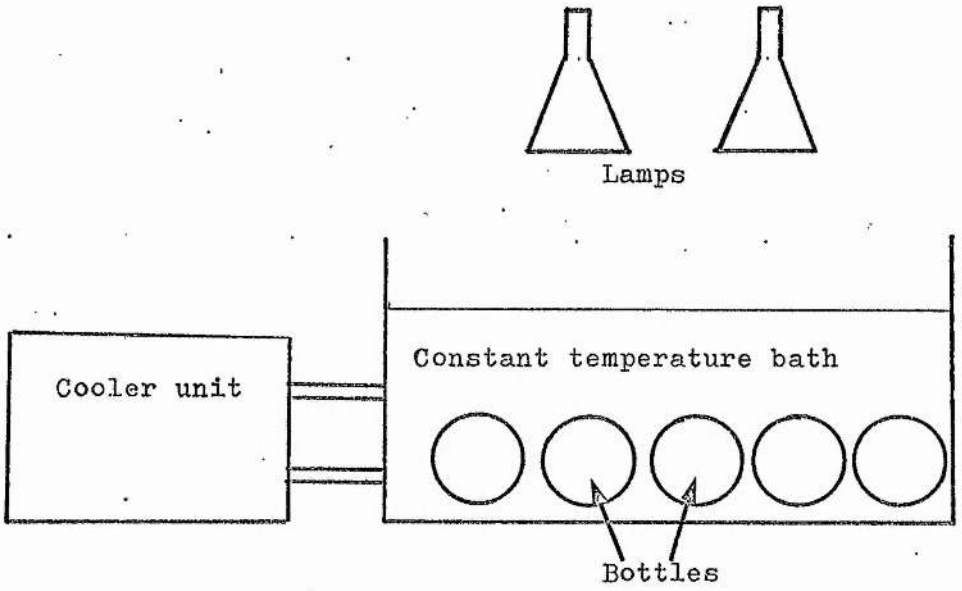


Figure 3.19

The apparatus used to determine the effect of incubation vessel size and amount of plant material per vessel on the pH change in the incubating fluid. The bottles were placed on their sides, in two rows of five, on the bottom of the water bath.

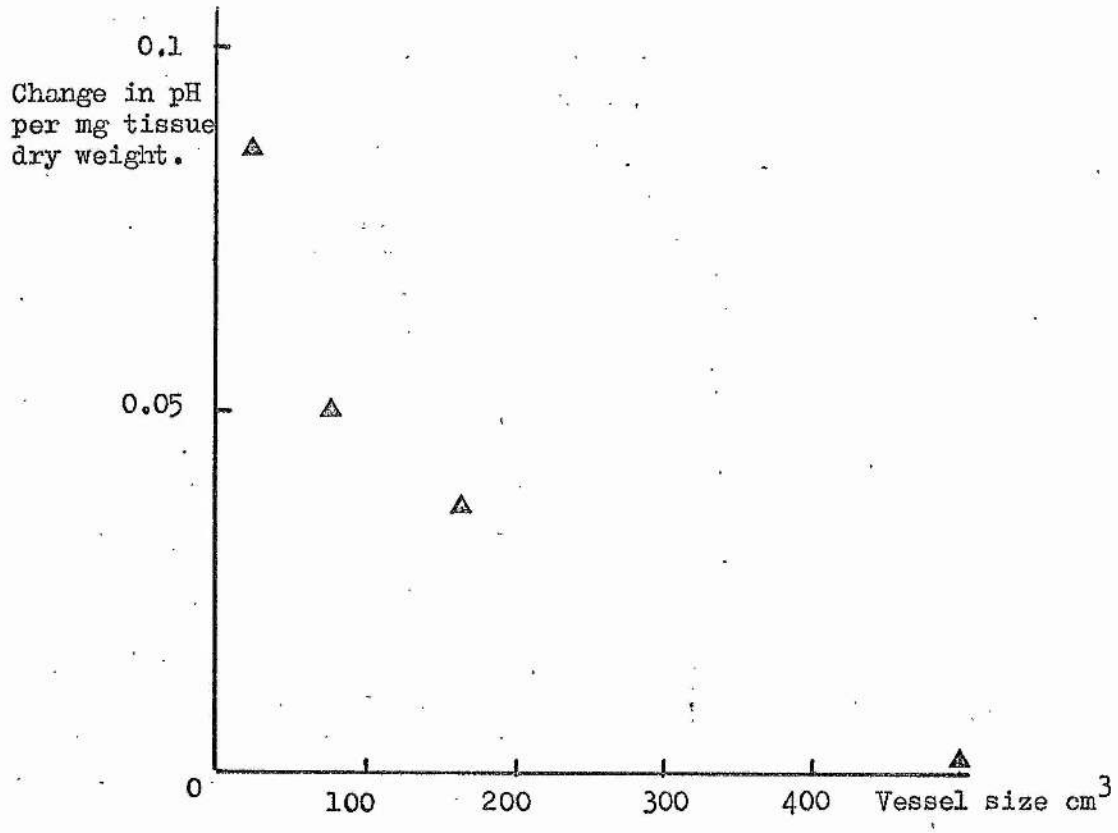


Figure 3.20

The relationship between the size of the incubation vessel (cm^3) and the pH change caused by leaves of P. perfoliatus during a 5 hour laboratory incubation.

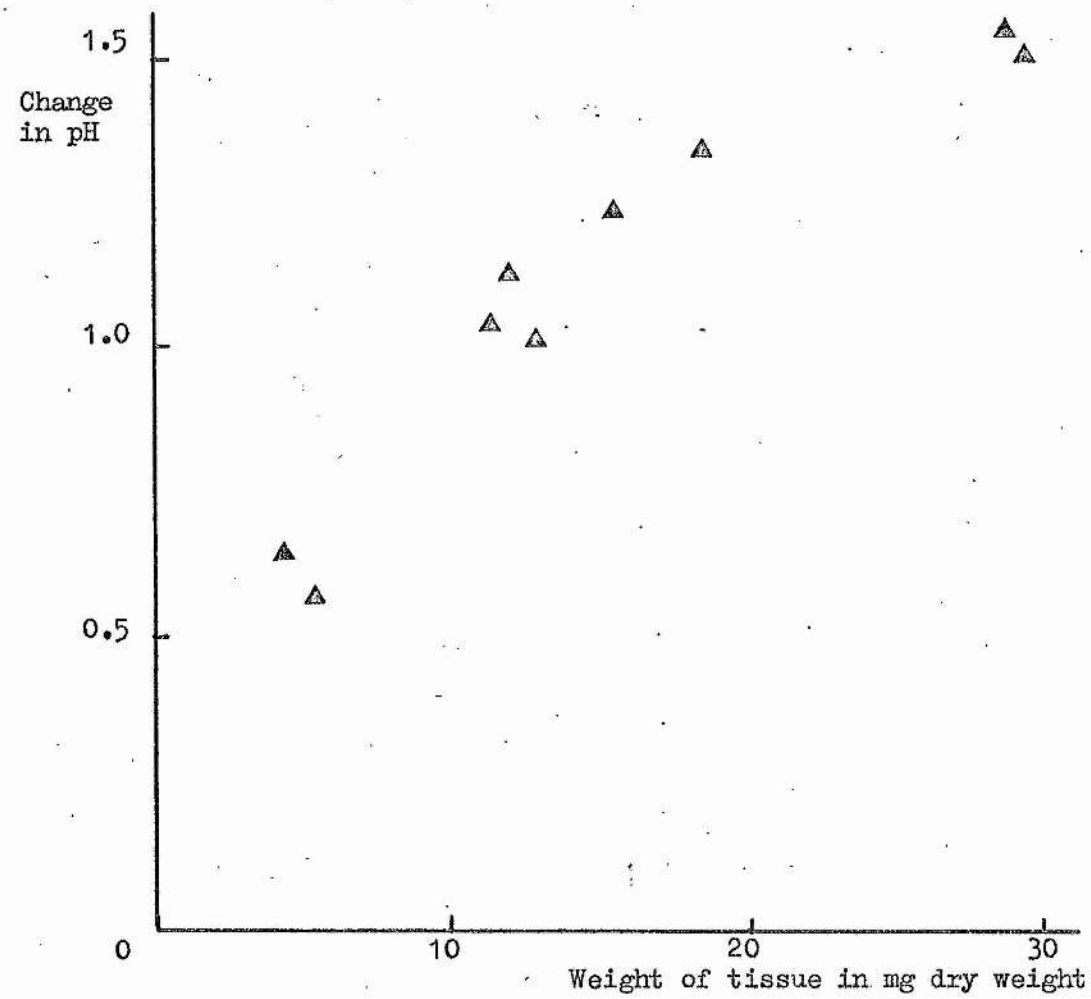


Figure 3.21

The relationship between the amount of leaf material (mg dry weight) and the pH change during a 5 hour laboratory incubation of P. perfoliatus in 25 cm^3 bottles.

the case of 500 cm^3 bottles this may be reduced to less than 1% (Figure 3.20). It can be further seen (Figure 3.21) that the pH increase in a given size of bottle varies considerably with the amount of material (mg dry weight) present.

These results suggest that the use of small amounts of plant tissue in large experimental enclosures would minimise the pH changes occurring during field experiments. Significant changes in pH will affect measured rates of photosynthesis as the proportions of the forms of inorganic carbon change. Therefore, for subsequent laboratory photosynthesis experiments the volume of incubating fluid in an enclosure was kept large in relation to the amount of plant material used, so that measurable pH changes did not occur.

CHAPTER FOUR

FLUID MOVEMENT, ITS EFFECT ON THE SUPPLY OF EXOGENOUS INORGANIC CARBON TO THE LEAF SURFACE OF AQUATIC MACROPHYTES

4.1 Fluid Movement Over a Leaf Surface

When a fluid flows past a planar surface, with a uniform velocity, the layer of molecules immediately in contact with the surface will remain stationary. The thermal energy of the fluid means its molecules are in a continuous state of random movement and there will be an interchange of molecules between different layers of fluid. This interchange will be responsible for transfer of momentum from one layer of molecules to another. Thus, molecules moving from the stationary layer to the adjacent layer will slow it down and molecules moving from this adjacent layer to the stationary layer will transfer their momentum to the planar surface causing a drag force. This process of net transfer of momentum from layer to layer occurs until a distance from the surface is reached such that adjacent layers are moving at the same velocity and no net momentum transfer takes place. This phenomenon is known as boundary layer formation, in which the velocity of the fluid increases with increasing distance from the surface until the velocity of the bulk fluid is reached.

Transfer of momentum will similarly be caused by the presence of intermolecular forces. In a gas the cross stream transfer of momentum by molecular movement will dominate due to the high thermal energy of molecules and inter-molecular forces will be insignificant. In

water, however, due to strong hydrogen bonding between molecules and much lower thermal energy, inter-molecular forces will predominate and cross stream transfer will be much less important.

Due to its polar nature water differs from air in its susceptibility to electrical charges on hydrophilic surfaces, such as protein, cellulose, and other macromolecules. A few layers of similarly orientated water molecules are formed next to the surface and an intermediate layer, in which disorder increases until the bulk water is reached, is also present. This layer of ordered water can be of the order of 1 to 2 nm thick (Drost-Hansen, 1969) and will be dependant on the nature of the surface. The properties of this ordered water are very different from those of the bulk water further away from the surface, and will have a much higher viscosity, a lower solubility, and hence a lower diffusivity for ions (Leyton, 1975).

In fluids the effectiveness of inter-molecular attraction and cross stream transfer in reducing velocity differences between adjacent layers of molecules is related to their dynamic viscosity. In fluid motion the density of the fluid must be taken into account and the dynamic viscosity is divided by the density to give the kinematic viscosity. Pressure tends to increase the dynamic viscosity of both liquids and gases but, within the range normally encountered,

the change will be negligible. The variation in density, dynamic viscosity and kinematic viscosity of air and water with temperature are given in Table 4.1 (Ede, 1967). This shows that the dynamic viscosity of air rises as the temperature increases, due to an increase in thermal motion with a consequent increase in cross stream transfer of momentum. The magnitude of this effect will be reduced by the decrease in density as temperature increases. In water, however, the effects of rising temperature are mainly confined to changes in density which reduce intermolecular forces and hence reduce dynamic viscosity. The density of air is more affected by temperature than the density of water and thus the kinematic viscosity of air varies more with temperature than water does.

The main differences between air and water are in the magnitude of their densities and viscosities. Thus, water has a density nearly 10^3 times that of air and its dynamic viscosity can be around 10^2 higher. This means that the kinematic viscosity of air will be 10 to 20 times larger than that of water.

The viscosity of water will be affected by the molar concentrations of solutes dissolved in it. In the case of sea water with a molarity of about 0·6, its viscosity (Table 4.1) is not very different from that of pure water. Thus, for the loch waters concerned, this effect need not be considered.

Table 4.1

The effect of temperature on the density, dynamic viscosity, and kinematic viscosity of both air and water. Values for sea water at one temperature are also given (Ede 1967).

Temperature ^{o}C	Density kg m^{-3}	Dynamic viscosity kg $m^{-1}s^{-1}10^{-5}$	Kinematic viscosity $m^{2}s^{-1}10^{-7}$	
0	1.3	1.72	133	
10	1.25	1.76	142	AIR
20	1.20	1.81	151	
30	1.16	1.86	160	
0	1000	179	17.1	
10	1000	131	13.1	WATER
20	1000	101	10.1	
30	1000	80	8.0	
20	1020	109	10.7	SEA WATER

The formation of the boundary layer will depend upon the presence of a surface. The change of the water velocity, with decreasing distance from the surface occurs slowly at first but as the distance becomes less the velocity quickly falls to zero. This produces the characteristic velocity profile of a boundary layer (Figure 4.2). The depth of the boundary layer is difficult to measure in practice but in theory it is the distance from the surface at which no further change in velocity takes place. As this change is asymptotic a compromise has to be reached and the depth is usually defined as the streamline along which the velocity reaches 95-99% of the value of the bulk fluid (Monteith, 1973; Curle and Davies, 1968; Cole, 1962).

The depth of the boundary layer will be related to the distance it occurs from the leading edge of a flat surface. For a thin plate such as a broad aquatic leaf, the boundary layer will have zero depth at the leading edge. As the distance downstream increases so the thickness of the boundary layer increases. This is a consequence of the increase in surface area producing a greater drag force. However, as the distance increases eventually a point is reached when conditions become unstable and the boundary layer is no longer laminar but becomes turbulent. The transition from a laminar to a turbulent boundary

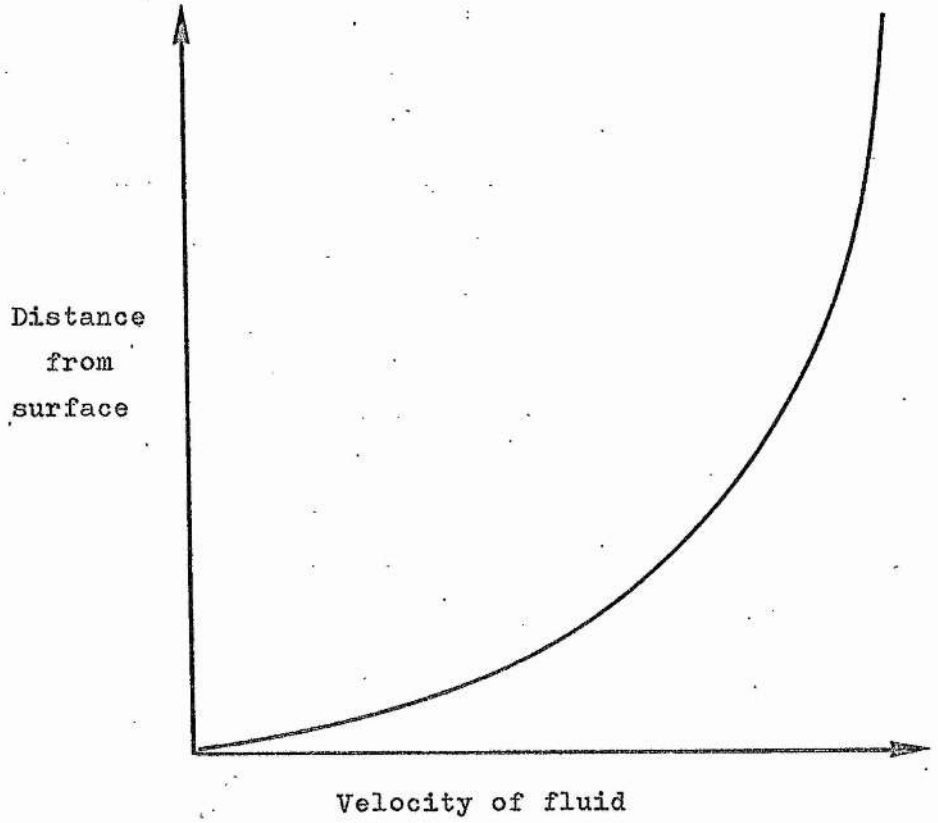


Figure 4.2

A representation of the velocity profile of a fluid at the boundary of a plane surface.

layer will not be sharp but will occur over a finite length of surface called the transition zone (Leyton, 1975). Although the boundary layer has become turbulent a laminar sub-layer will still persist (Figure 4.3) which decreases in depth as the turbulent boundary layer increases.

The change from a laminar boundary layer to a turbulent boundary layer over a surface will be dependant on the value of the Reynolds number (R_e) which is the ratio of the magnitude of pressure forces to viscous forces in a fluid (Curles and Davies, 1968), i.e.:

$$R_e = \frac{\rho \cdot U^2}{\mu \cdot \frac{U}{L}} = \frac{UL}{V}$$

where V = kinematic viscosity

U = Bulk velocity of fluid

L = Distance from leading edge of surface.

When the ratio is small, viscous forces predominate and flow tends to remain laminar, but when the ratio increases beyond a critical value, pressure (inertial) forces predominate and flow becomes turbulent. For water and air over the range of temperature from 0-30°C the value of the kinematic viscosity (V) remains relatively constant (Table 4.1) and the onset of a turbulent boundary layer will depend mainly upon the bulk fluid velocity (U) and the length of surface involved (L). The variation in Reynolds number with length of

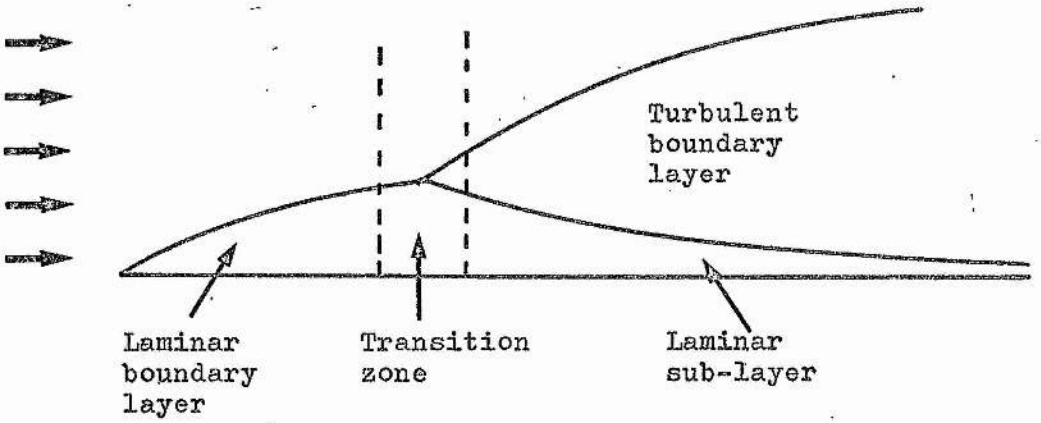


Figure 4.3

A representation of the laminar boundary layer, turbulent boundary layer, and laminar sub-layer formed in a fluid, with uniform velocity, over a smooth planar surface (Leyton, 1975).

surface and fluid velocity, for air and water, are given in Tables 4.4a and b at $20^{o}C$.

Critical Reynolds numbers above which turbulent flow would be produced, are of the order of 3×10^{6} (Leyton, 1975) for smooth surfaces.

Therefore $UL = 3 \times 10^{6} \times V$

and for water at $20^{o}C$ ($V = 10{\cdot}1 \times 10^{-7}$)

$$UL \leq 3{\cdot}03.$$

A suitable upper size limit for the length of leaf, of a broad-leaved Potamogeton sp. would be 0·25 m and this would require a bulk velocity of about 12 ms^{-1} (or 43·2 kmh^{-1}) for turbulent boundary layer formation. If the bulk flow is more turbulent than laminar then the critical Reynolds number would be as low as 5×10^{5}.

Therefore $UL \leq 0{\cdot}505$ and for the upper limit on leaf size of 0·25 m the bulk velocity would have to be about 2 ms^{-1} (or 7·2 $km.h^{-1}$) to exceed the critical Reynolds number..

As the kinematic viscosity of air is 10 to 20 times that of water (Table 4.1) it is apparent that for a leaf of the same size in air the Reynolds numbers will be lower (Table 4.4) and wind velocities will have to be high to produce turbulent flow over a smooth surface.

Table 4.4a

The variation of Reynolds number, Re, with the distance (L) from the leading edge of a plane surface, and the bulk fluid velocity (U) of air calculated from $Re = \frac{UL}{V}$ where V = kinematic viscosity of air at 20°C ($V = 1.51 \times 10^{-5} m^2 s^{-1}$).

		L (m)					
		0.25	0.15	0.10	0.05	0.02	0.01
	0.01	1.7×10^2	99	66	33	13.3	6.6
	0.06	9.9×10^2	6.0×10^2	4.0×10^2	2×10^2	79	40
$U(ms^{-1})$	0.1	1.7×10^3	9.9×10^2	6.6×10^2	3.3×10^2	1.3×10^2	66.2
	0.25	4.1×10^3	2.5×10^3	1.7×10^3	8.3×10^2	3.3×10^2	1.7×10^2
	0.5	8.3×10^3	5.0×10^3	3.3×10^3	1.7×10^3	6.6×10^2	3.3×10^2
	1.0	1.7×10^4	9.9×10^3	6.6×10^3	3.3×10^3	1.3×10^3	6.6×10^2

Table 4.4b

The variation of Reynolds number, Re. with the distance (L) from the leading edge of a plane surface and the bulk fluid velocity (U) calculated from $Re = \frac{UL}{V}$ where V = kinematic viscosity of water at 20°C. ($V = 10.1 \times 10^{-7} m^2 s^{-1}$).

	L (m)					
	0.25	0.15	0.10	0.05	0.02	0.01
0.01	2.5×10^3	1.5×10^3	9.9×10^2	5.0×10^2	2.0×10^2	9.9
0.06	1.5×10^4	8.9×10^3	5.9×10^3	3.0×10^3	1.2×10^3	5.9×10^2
0.1	2.5×10^4	1.5×10^4	9.9×10^3	5.0×10^3	2.0×10^3	9.9×10^2
$U(ms^{-1})$						
0.25	6.1×10^4	3.7×10^4	2.5×10^4	1.2×10^4	5.0×10^3	2.5×10^3
0.5	1.2×10^5	7.4×10^4	5.0×10^4	2.5×10^4	9.9×10^3	5.0×10^3
1.0	2.5×10^5	1.5×10^5	9.9×10^4	5.0×10^4	2.0×10^4	9.9×10^3

Therefore, submerged leaves of broad-leaved pondweeds, in the lake environment, are more likely to have a laminary boundary layer present than a turbulent boundary layer. In streams on the other hand, with more turbulent flow, higher bulk velocities, and longer, linear leaved pondweeds, turbulent boundary layers might be expected to be formed over part of the surface of the leaves.

The depth (d) of the laminar boundary layer over a smooth flat surface is usually given as

$$d = 5L\ (R_{eL})^{-1/2} \quad \text{(Blassius, 1908)}$$

where L = the distance from the leading edge and R_{eL} is the Reynolds number at that distance. The value of 5 is only an approximation (Leyton, 1975) and other slightly different values have been calculated. The equation is of more use in the form

$$d = 5.V^{1/2}.\ L^{1/2}.\ U^{-1/2}$$ (where V = kinematic viscosity)

and values of d for different values of L and U, both for air and water, have been calculated (Table 4.5a and b). Thus, the depth decreases as the square root of the bulk velocity (U) and increases as the square of the distance from the leading edge (L). For leaves of similar sizes and at similar bulk fluid velocities the depth of the laminary boundary layer in water will be smaller than in air. It will, in fact, be proportional

Table 4.5a

The effect of surface length (L) and bulk fluid velocity, on the depth of the laminar boundary layer of air, calculated from $d = 5. V^{\frac{1}{2}}. L^{\frac{1}{2}}. U^{-\frac{1}{2}}$ and expressed in m x 10^{-3} at 20°C.

	L (m)					
	0.25	0.15	0.10	0.05	0.02	0.01
0.01	97	75	61	43	28	19
0.06	39	31	25	18	11	7.9
0.1	30	24	19	14	8.7	6.1
U(ms^{-1})						
0.25	19	15	12	8.7	5.5	3.9
0.5	13	11	8.7	6.1	3.9	2.7
1.0	9.7	8	6.1	4.3	2.7	1.9

Table 4.5b

The effect of surface length (L) and bulk fluid velocity on the depth of the laminar boundary of water, over a plane surface, calculated from $d = 5.V^{\frac{1}{2}}.\ L^{\frac{1}{2}}.\ U^{-\frac{1}{2}}$ and expressed in metres x 10^{-3} at $20^{o}C$.

	L (m)					
	0.25	0.15	0.10	0.05	0.02	0.01
0.01	25	20	16	11.2	7.1	5.0
0.06	10	8.0	6.5	4.6	2.9	2.1
0.1	8.0	6.2	5.0	3.6	2.3	1.6
U(ms^{-1})						
0.25	5.0	3.9	3.2	2.3	1.4	1.0
0.5	3.6	2.8	2.3	1.6	1.0	0.71
1.0	2.5	2.0	1.6	1.1	0.71	0.50

to the square root of their kinematic viscosities such as

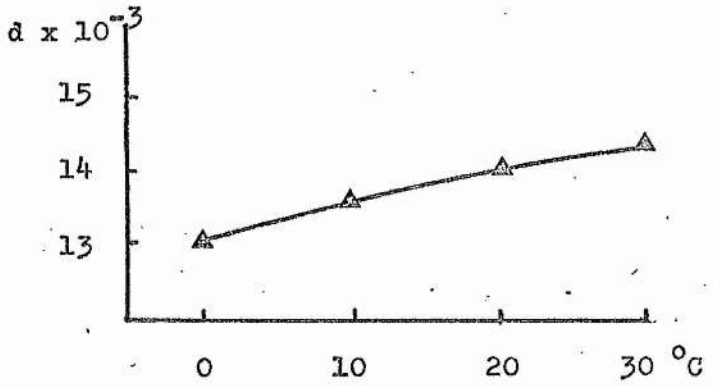
$$\frac{d_{Air}}{d_{Water}} = \frac{\sqrt{\nu_{Air}}}{\sqrt{\nu_{Water}}} = \frac{\sqrt{151 \times 10^{-7}}}{\sqrt{10{\cdot}1 \times 10^{-7}}} = 3{\cdot}87$$

The kinematic viscosity of a fluid is temperature dependant and the thickness of the laminary boundary layer will be similarly affected. The dependance of thickness of the laminary boundary on temperature is shown in Figure 4.6a and b for air and water. It can be seen that the laminar boundary layer increases with temperature for air and decreases with temperature for water.

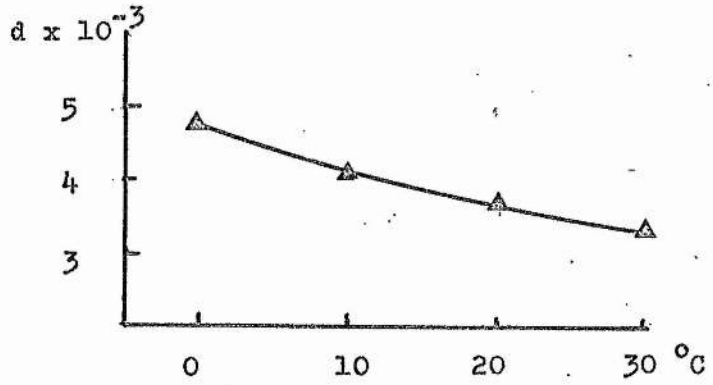
Although laminary boundary layers might be expected to predominate for broad leaves both in the terrestrial and aquatic systems the formation of a turbulent boundary must be considered. The depth of this layer is given as

$$d_T = 0{\cdot}376_{L_T} \cdot (Re_{L_T})^{-1/5}$$

where L_T is the distance from the start of turbulence and R_{L_T} is the corresponding Reynolds number based upon this. The effect of both L_T and U on the thickness of d_T for air and water are calculated and given in Tables 4.7a and b. This shows that the thickness increases more rapidly with length than the laminar boundary layer and that higher bulk fluid velocities will reduce it less.



a/ Air.



b/ Water.

Figure 4.6

The effect of temperature on the thickness (d) of the laminar boundary layer (metres) over a plane surface in air (a) and water (b).

Table 4.7a

The variation of the depth (metres x 10^{-3}) of the turbulent boundary layer in air with its length (L_T) and the bulk fluid velocity (U) as calculated from $d_T = 0.376 . V^{\frac{1}{5}} . L_T^{\frac{4}{5}} . U^{-\frac{1}{5}}$ at 20°C.

	L (m)					
	0.25	0.15	0.10	0.05	0.02	0.01
0.01	33.8	22.5	16.3	9.3	4.5	2.6
0.06	23.7	15.8	11.4	6.5	3.1	1.8
0.1	21.3	14.3	10.3	5.9	2.8	1.6
U(ms^{-1})						
0.25	17.9	11.9	8.5	4.9	2.4	1.4
0.5	15.5	10.3	7.4	4.3	2.0	1.2
1.0	13.4	8.9	6.5	3.7	1.8	1.0

Table 4.7b

The variation of the depth (m x 10^{-3}) of the turbulent boundary layer in water with its length (L_T) and the bulk fluid velocity (U), as calculated from $D_T = 0.376\ V^{\frac{1}{5}}\ L_T^{\frac{4}{5}}\ U^{-\frac{1}{5}}$ (Leyton 1975) at 20°C.

	L (m)					
	0.25	0.15	0.10	0.05	0.02	0.01
0.01	19.7	13.1	9.46	5.44	2.61	1.50
0.06	13.8	9.2	6.61	3.80	1.82	1.05
0.1	12.4	8.3	5.97	3.43	1.65	0.95
0.25	10.4	6.9	4.97	2.86	1.37	0.79
U(ms^{-1})						
0.5	9.0	6.0	4.33	2.49	1.19	0.69
1.0	7.8	5.2	3.77	2.16	1.04	0.60

However when a turbulent boundary layer is produced over a surface there will still be a laminar sub-layer present (Leyton, 1975)(Figure 4.3). The depth of this laminar sub-layer (d_{SL}) is given as

$$d_{SL} = \frac{58\ LT}{(Re_{LT})^{0.9}} \text{ which approximates to } d_{SL} = \frac{58V}{U},$$

giving the values calculated in Table 4.8 for different values of U for air and water. This shows that the depth is directly proportional to the kinematic viscosity and the ratio of the depth of the laminar sub-layer in air to that in water will be $\frac{V_{air}}{V_{water}} = 15.$

The preceding analysis of fluid movement over smooth plane surfaces will require certain assumptions for its meaningful application to the real situation of a plant leaf.

The assumption that a leaf presents itself with its main axis parallel to the direction of flow is the worst case. This allows the length of the leaf to correspond to the length of the plane surface (L) but this will not always be the case as it is quite possible for the leaf to be normal to the direction of flow. However, it would also be likely that as bulk velocity of water increases for the leaf will be dragged round to point downstream. The same argument will apply to the inclination of the plane of the leaf to the direction of the flow. This assumption will not affect the expected change from laminar to turbulent flow as shorter values of surface length (L) will require

Table 4.8

Approximate values of the depth of the laminar sub-layer (d_{SL}) for air and water, at different bulk fluid velocities (μ), at 20^0C.

U ($m.s^{-1}$)	d_{SL} ($m \times 10^{-3}$)	
	Air	Water
0.01	87.6	5.86
0.06	14.6	0.98
0.1	8.76	0.59
0.25	3.50	0.23
0.5	1.75	0.12
1.0	0.88	0.06

higher bulk velocities and thus for a given situation the transition from laminar to turbulent flow will be underestimated.

The shape of a leaf is quite different to a rectangular plane as the leading edge will be pointed, not straight and not normal to one direction of flow. This difference in shape will cause a greater edge effect and a proportionally lower average depth of the boundary layer when compared to a rectangular surface of the same area. The effect of leaf shape on boundary layers has been demonstrated with cotton leaves (Baker and Myhre, 1969). They showed that deeply-lobed leaves were found to have thinner boundary layers than normal leaves, but failed to demonstrate any effect on carbon dioxide fixation as the leaf boundary layer resistance under field conditions was small compared to the total diffusive resistance.

The leaf will not be absolutely flat but unless severely crinkled it is unlikely to restrict laminar boundary layer formation under normal conditions. The surface roughness will have an effect on fluid flow, with increasing roughness tending to promote the onset of turbulence. It has been indicated that surface structures such as leaf-hairs or wax filaments (Sanchez-Diaz, Hesketh and Kramer, 1972) should affect the boundary layer resistance to diffusion of gases and water vapour. Direct measurement of unstirred layers on rabbit cornea and contact lenses (Green and Octovi,

1970), by visual observation of small polystyrene latex spheres, showed that under unstirred conditions the thickness on the cornea was 350 μm and that on the contact lens 15 μm. Vigorous stirring reduced these unstirred layers to 65 μm and 20 μm respectively. That is, when unstirred the thickness on the cornea was 2·3 times that on the contact lens, but when stirred this ratio increased to over 3·2.

They concluded that the relative smoothness of the artificial surface compared to the biological membrane was responsible for the difference. This difference between the stirred and the unstirred situation may be considered in terms of the laminar sub-layer. At low bulk fluid velocities the 'peaks' of roughness may be submerged in the laminar sub-layer and the surface appears smooth while at higher fluid velocities the laminar sub-layer decreases in depth and the 'peaks' of roughness emerge and the surface is effectively rougher. It would seem reasonable to consider the plant leaf as behaving more like a cornea than the artificial surface of the contact lens.

Implicit in the analysis of fluid flow over a smooth plane surface is that it remains rigid. A plant leaf is flexible and it will be prone to flapping if the fluid forces are high enough and the leaf is not very rigid. However, Parlange, Waggoner and Heichel (1971)

concluded that the mild flutter of leaves in natural wind has no effect on the average boundary layer thickness since it had no effect in the laboratory under rather extreme conditions. In the loch environment with lower fluid velocities there is unlikely to be any significant flapping of leaves or any consequent reduction in boundary layer thickness.

Consideration of fluid movement around a leaf must take into account convection currents set up by temperature differences between the leaf surface and the fluid. The solar irradiance incident upon the leaf surface will heat it and the energy absorbed will be dissipated to the surrounding fluid. This dissipation of heat energy will be proportional to the incident energy and a high proportion of solar energy is in the red to infra red end of the spectrum. This tends to be selectively filtered out by the water and underwater light regimes will cause less heating of leaves under similar conditions. The thermal conductivity of water is over 20 times that of air (Table 4.9) and it will therefore be much easier for the aquatic leaf to lose heat. That is, the temperature rise required for the same heat loss will be lower in water than in air. The formation of convection currents will be proportional to the change in density of the fluid caused by the temperature increase. Therefore, loss of the same amount of heat in air will produce larger convection currents. The effects of convection currents will

Table 4.9

The values of the thermal conductivity of air and water as affected by temperature (Ede 1967). ($W\ m^{-1}\ {}^{0}C^{-1}$).

Temperature ^{0}C	Air	Water
0	0.0241	0.55
10	0.0249	0.58
20	0.0257	0.60
30	0.0265	0.62

depend upon the bulk fluid velocity. When this is zero the convection forces will dominate, but as the bulk fluid velocity increases a level will be reached such that removal of heat from the leaf is accomplished with negligible density changes.

In conclusion, therefore, a theoretical analysis of fluid flow over leaf surfaces can give quantitative estimates of the laminar boundary layers that are formed. The supply of inorganic carbon to the leaf surface will depend upon its ability to pass through these layers and therefore the following section examines the diffusion of carbon dioxide.

4.2 The Molecular Diffusion of Carbon Dioxide

The rates of diffusion of carbon dioxide in air and water are significantly different, with the diffusivities being $1{\cdot}47 \times 10^{-1}$ and $1{\cdot}71 \times 10^{-5}$ $cm^2 . s^{-1}$ (Table 4.10) respectively at $20^{o}C$. The flux of a diffusing substance is given by Fick's first law of diffusion: Flux = Diffusivity x Concentration gradient. Therefore, in comparable situations where the concentration gradients were similar the flux of carbon dioxide in water would be 10^4 less than that in air.

However, the diffusivity considered here is the molecular diffusion coefficient which is small compared to the process of eddy diffusion. When eddy diffusion takes place the dissolved carbon dioxide will be moved by the fluid itself and this may be several orders of magnitude faster than molecular diffusion. That means that the flux of carbon dioxide will be controlled wherever molecular diffusion takes place. This will occur across the laminar boundary layer and Fick's law can be applied to this:

$$F_{CO_2} = D_{CO_2} \times \frac{(CO_2)bulk - (CO_2)leaf}{d}$$

where F_{CO_2} = flux of carbon dioxide

D_{CO_2} = diffusivity of carbon dioxide

$(CO_2)bulk$ = concentration of CO_2 in bulk solution

$(CO_2)leaf$ = concentration of CO_2 at the leaf surface

d = depth of the laminary boundary layer.

Table 4.10

The effect of temperature on the diffusion coefficients of carbon dioxide in air and water. (International Critical Tables 1929 and Monteith 1973).

	Diffusion coefficient ($cm^2\ s^{-1}$)	
Temperature ^{o}C	Air	Water
10	1.39×10^{-1}	1.46×10^{-5}
15	1.42×10^{-1}	1.46×10^{-5}
20	1.47×10^{-1}	1.71×10^{-5}

This can also be applied to the internal diffusion path:

$$F_{CO_2} = D \times \frac{(CO_2)leaf - (CO_2)chl}{i}$$

where $(CO_2)chl$ = concentration of CO_2 at the chloroplast

i = internal diffusion path.

As the flux of CO_2 will be the same one in each case considered:

$$F_{CO_2} = \frac{D}{d}\left((CO_2)bulk - (CO_2)leaf\right) = \frac{D}{i}\left((CO_2)leaf - (CO_2)Ch\right.$$

The magnitude of i can be estimated by sectioning leaves of Potamogeton sp. and measurement of the dimensions of the cells in the section. With the exception of the mid-rib and smaller lateral ribs the surface is three cells thick. The leaf thickness is of the order of 5×10^{-5}m. and hence the half leaf thickness of about $2{\cdot}5\ 10^{-5}$m. This is significantly less than any laminar boundary layer over the leaf (Table 4.5a and b), and only at high water velocities (1 ms^{-1}) does the thickness of the laminar sub-layer approach this (Table 4.8). The laminar boundary layer might be in the range 2×10^{-3} to 16×10^{-3}m which is more than an order of magnitude greater than half leaf thickness. The molecular diffusion through the laminar boundary layer will be significant in controlling the rate of supply of carbon dioxide to the aquatic leaf.

For a given concentration difference the flux of carbon dioxide will be proportional to $\frac{D}{d}$ and the ratio

of fluxes in air and water will be $\frac{D_{CO_2}(AIR)}{d_{AIR}}$.

$\frac{d_{H_2O}}{D_{CO_2}(H_2O)}$. For similar sized leaves under similar conditions this will be $2{\cdot}2 \times 10^3$. That is the potential carbon dioxide flux in air will be $2{\cdot}2 \times 10^3$ times the flux in water at the same values of leaf size, temperature, bulk carbon dioxide, and bulk fluid velocity.

Therefore, a consideration of laminar boundary layers and diffusion of carbon dioxide across them indicates that this process might be a significant limiting factor for photosynthesis by broad leaved pondweeds. The remainder of this chapter, therefore, investigates the effect of different flow conditions on the measured rate of ^{14}C incorporation by whole leaves and leaf discs in the laboratory. First, however, the laboratory light regime was calibrated and the rates of ^{14}C incorporation, by samples of the plant material to be used in the flow experiment, were measured at different light intensities in the following series of experiments.

4.3 The Effect of Light Intensity on the ^{14}C Incorporation by Leaf Discs of P. praelongus in the Laboratory

The experiments on the effect of flow and period of incubation on the ^{14}C incorporation by P. praelongus were performed under relatively high light intensities from an overhead light system in the laboratory. To determine the effect of the intensity upon the interpretation of the results of these experiments the intensity of the overhead light system was varied and its effect on the rate of ^{14}C incorporation observed. The light intensities were measured with an I.S.C.O. spectroradiometer and a Lambda quanta meter.

The light system Three 1500 watt quartz halogen strip lamps were mounted in the overhead illumination system shown in Figure 4.11. An electric fan provided a horizontal flow of air to cool the lamps and the aluminium reflectors directed the light downwards through the glass-bottomed water bath. The unwanted infrared radiation was removed by a continuously replenished 7 cm deep layer of water in the bottom of the tank.

Immediately beneath the bath a rack was fitted so that one or more filters could be fitted. These were of a neutral density type, and as such will not change the relative proportions of the wavelengths of the light. The whole apparatus was surrounded by a black curtain to exclude any other light. The system enabled light to be provided to any chosen photosynthesis experiment on the

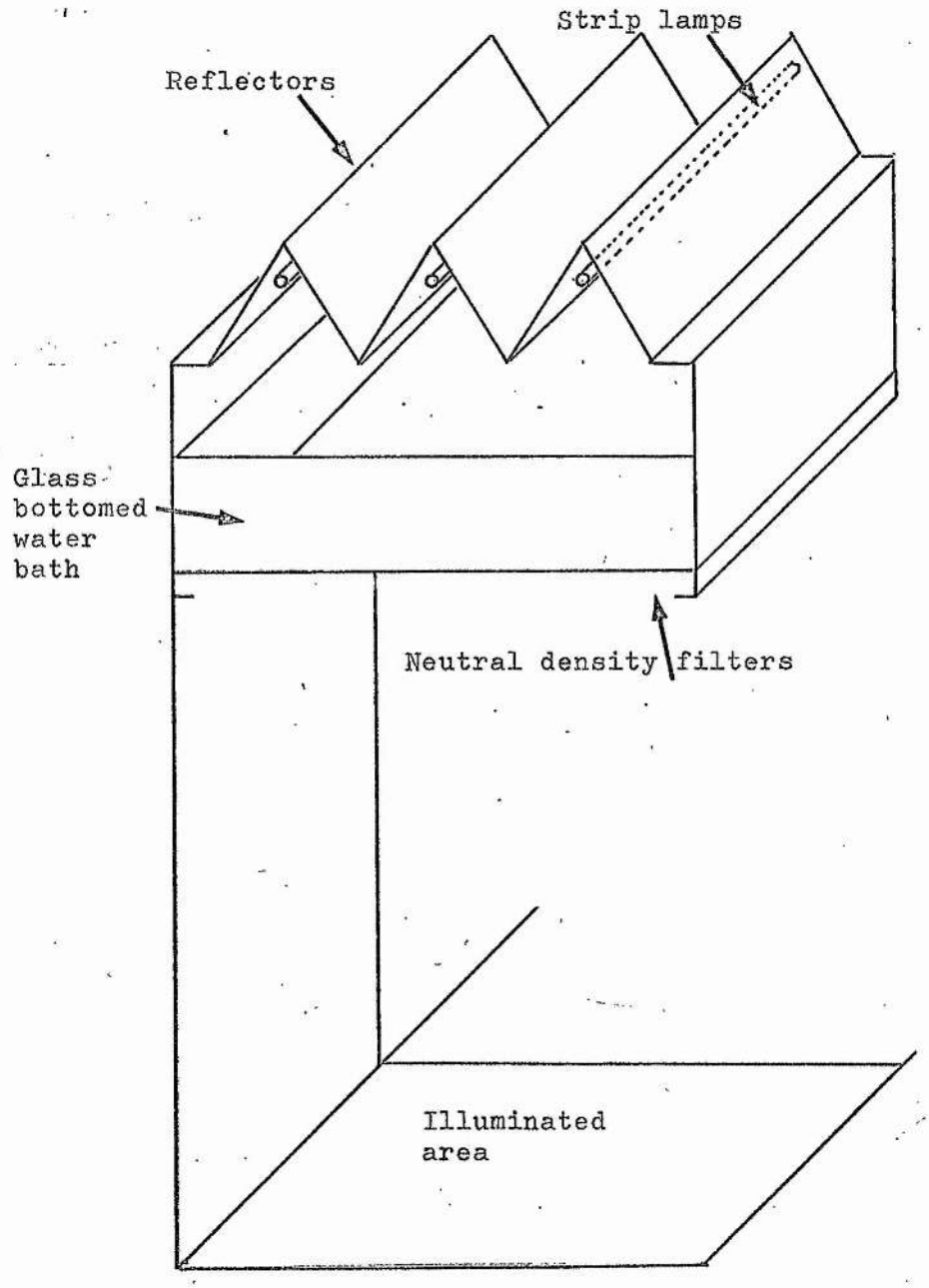


Figure 4.11

The overhead light system used to provide illumination for the flow and light experiments. Three 1.5 kw quartz halogen strip lamps are mounted in reflectors and cooled with an electric fan. The light from these is passed through a 7 cm deep water bath with a glass bottom. Beneath this neutral density filters are mounted in a rack and a curtain surrounded the apparatus to exclude stray light.

laboratory bench beneath it.

The measurement of light An I.S.C.O. spectroradiometer was placed beneath the lights so that its detector occupied the same position as the experimental enclosures. By moving the detector around from this position it was possible to confirm that the light field was uniform, provided a neutral density filter was fitted to diffuse the light. Therefore, all photosynthesis experiments were carried out with one neutral density filter added.

The spectroradiometer was then set at intervals of 25 nm wavelength and the readings obtained at these wavelengths (Table 4.12). The instrument was calibrated, in a darkroom, by measuring the spectrum of a standard lamp and calculating a calibration factor from the ratio of the actual reading to the known intensity (Figure 4.13). The distribution of the irradiance with wavelength provided by the overhead light system was then calculated (Table 4.14) and is shown in Figure 4.15. This shows that the light provided by this system is deficient in the blue region, with a bias toward the red end of the spectrum, when compared to sunlight. The drop beyond 700 nm shows the absorption of the water bath and its glass bottom.

It is also possible to integrate the area under this curve, between the limits of photosynthetically active radiation (390 nm - 750 nm), by summing the average number of quanta for each 25 nm bandwidth.

Table 4.12

The results of a spectral intensity analysis using a spectro-radiometer on the overhead quartz halogon strip light system.

Wavelength	Reading	Full scale deflection	Result	Calibration factor	($\mu W.cm^{-2}.nm^{-1}$) Intensity
400	1.4	0.3	0.14	30.35	4.27
425	8.8	1.0	0.88	4.87	4.29
450	1.95	3.0	1.95	3.01	5.87
475	3.1	10.0	3.1	2.55	7.91
500	5.0	10.0	5.0	1.86	9.3
525	6.7	10.0	6.7	1.795	12.03
550	1.05	30.0	10.5	1.44	15.12
575	1.33	30.0	13.3	1.32	17.56
600	1.58	30.0	15.8	1.26	19.91
625	1.8	30.0	18.0	1.15	20.7
650	2.0	30.0	20.0	1.12	22.4
675	2.23	30.0	22.3	1.02	22.75
700	2.45	30.0	24.5	1.02	24.99
725	2.22	30.0	22.2	1.07	23.75
750	1.65	30.0	16.5	1.26	20.79

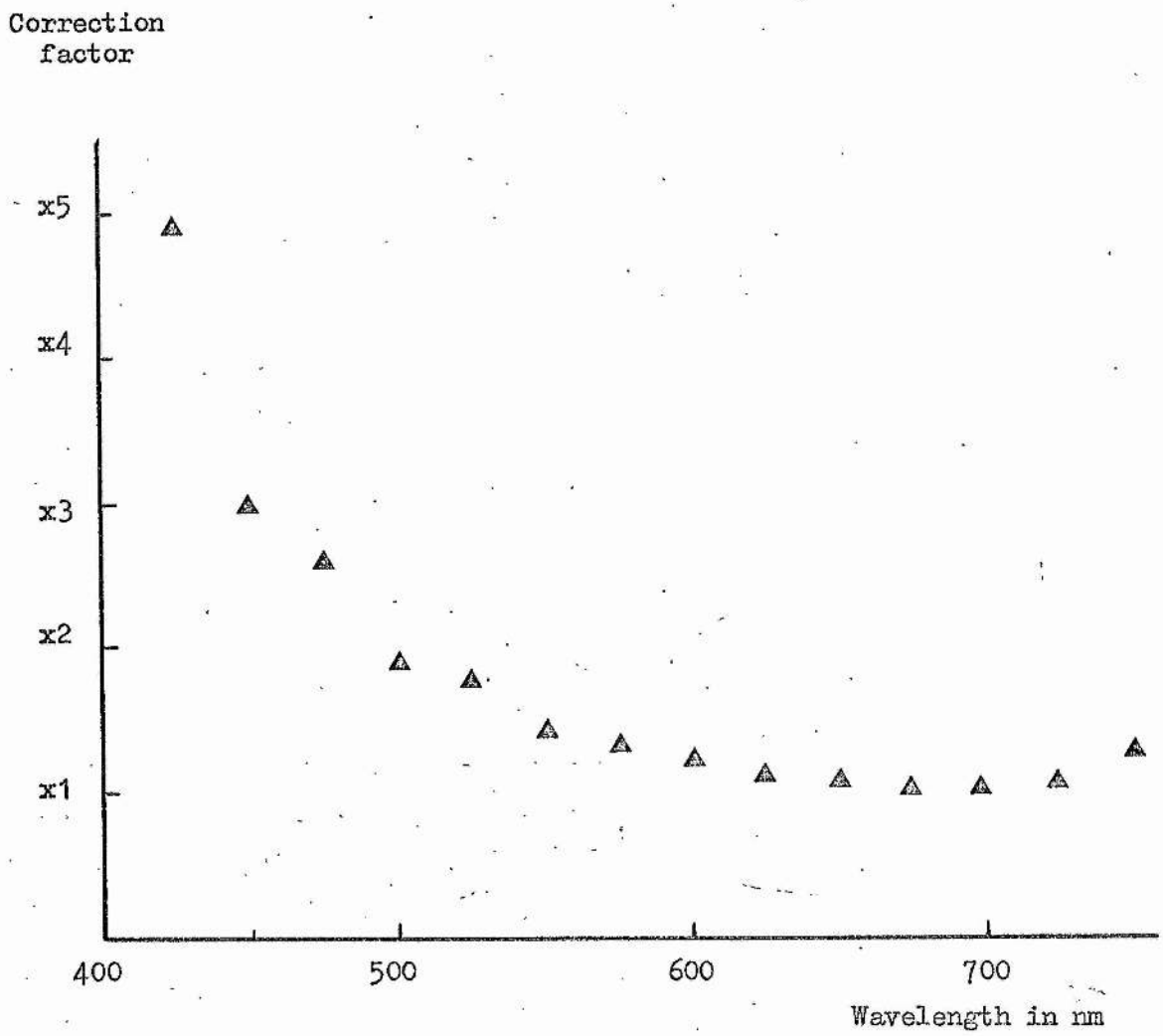


Figure 4.13

The calibration curve for the I.S.C.O. spectroradiometer with the ordinate giving the factor by which the reading on the meter should be multiplied to obtain the intensity in micro-watts per cm^2 per nm.

Table 4.14

The calculation of photosynthetically active radiation (390-750nm) in units of micro-einsteins per metre^2 per second, from the results of a spectral intensity analysis using an I.S.C.O. spectroradiometer.

Wavelength nm	Intensity ergs sec^{-1} m^{-2} per nm	Bandwidth nm	Intensity over bandwidth ergs.sec^{-1} m^{-2} $x10^6$	Ergs per einstein $x10^6$	Einsteins $x10^{-6}$ $m^{-2}sec^{-1}$
400	4.27	22.5	9.61	3.0	3.2
425	4.29	25	10.73	2.81	3.82
450	5.87	25	14.68	2.65	5.54
475	7.91	25	19.78	2.53	7.82
500	9.3	25	23.25	2.39	9.73
525	12.03	25	30.08	2.27	13.25
550	15.12	25	37.8	2.17	17.42
575	17.56	25	43.9	2.08	21.11
600	19.91	25	49.78	1.99	25.02
625	20.7	25	51.75	1.91	27.09
650	22.4	25	56.0	1.84	30.44
675	22.75	25	56.88	1.76	32.32
700	24.99	25	62.48	1.7	36.75
725	23.75	25	59.38	1.64	36.21
750	20.79	12.5	25.99	1.6	16.24
					285.96

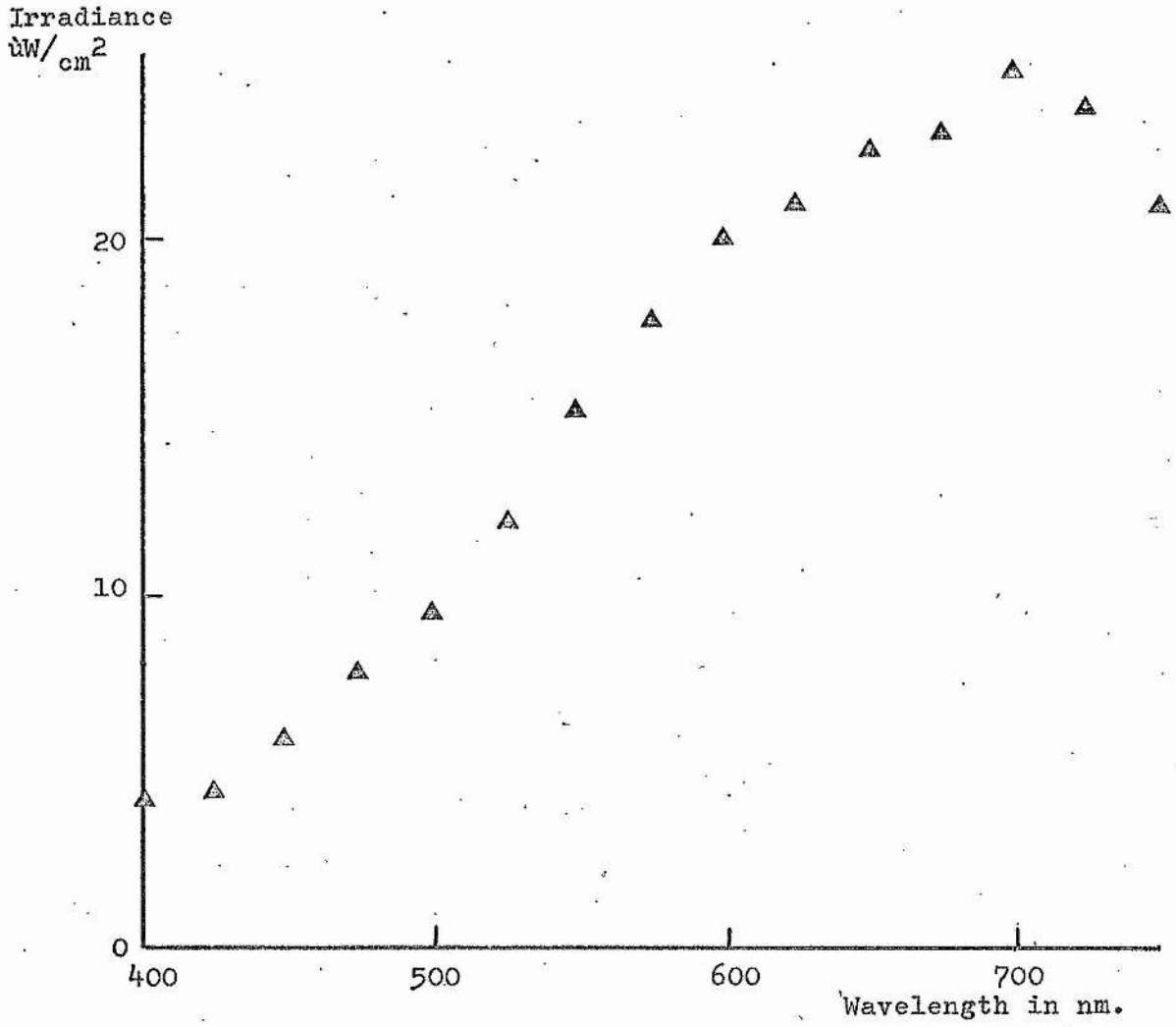


Figure 4.15

The distribution of the irradiance of quartz halogen strip lights along the band width of photosyntheticaly active radiation.

Thus, at 500 nm the light intensity is measured as $9{\cdot}3 \times 10^5$ erg $m^{-2}sec^{-1}nm^{-1}$ ($=9{\cdot}3$ w $cm^{-2}nm^{-1}$) and the energy in the 25 nm bandwidth 487·5-512·5 will be $23{\cdot}25 \times 10^6$ erg $m^{-2}sec^{-1}$. If this value is divided by the average energy per einstein of quanta (i.e. at 500 nm) then the number of einsteins can be obtained. These are then summed for each of the 25 nm bandwidths to give the number of einsteins m^{-2} sec^{-1} provided by the light system from 390-750 nm (Table 4.14).

For the different light intensity treatments, however, the irradiances were measured directly in microeinsteins, for 390-750 nm, by the use of a Lambda quanta meter. The results of this measurement for the different filter combinations L_1 - L_7 are given in Table 4.16.

The measurement of ^{14}C incorporation Shoots of *P. praelongus* were collected fresh from Loch Drumore, by aqualung diving, and stored in loch water until required for experimentation. Fourteen 2·0 cm diameter discs were cut, each from a fresh leaf, immediately prior to requirement in an experiment. These were washed in the incubating medium 2×10^{-3} M $KHCO_3$ and placed in the two replicate 1·8 l Kilner jar enclosures (Figure 4.17). Each disc was held by a hair grip so that all the discs in a jar were all in a row with the discs in a plane normal to the incident light. These operations were carried out in subdued lighting and care was taken to prevent rough handling of the discs.

Table 4.16

The measurement of the photosynthetically active radiation by a Lamda quanta meter, in micro-einsteins per m^2 per second, for the different light treatments used to investigate the effect of light intensity on the ^{14}C incorporation by P. praelongus. The different light intensities, L_1 - L_7, are produced by the use of various combinations of neutral density filters with the overhead light system (Figure 4.11)

Filter combination	Reading	Range	Light intensity (micro-einsteins per m^2 per sec)
L_1	3.6	1,000	360.0
L_2	1.6	300	160.0
L_3	1.03	300	103.0
L_4	4.2	100	42.0
L_5	9.3	10	9.3
L_6	5.1	10	5.1
L_7	2.85	3	2.85

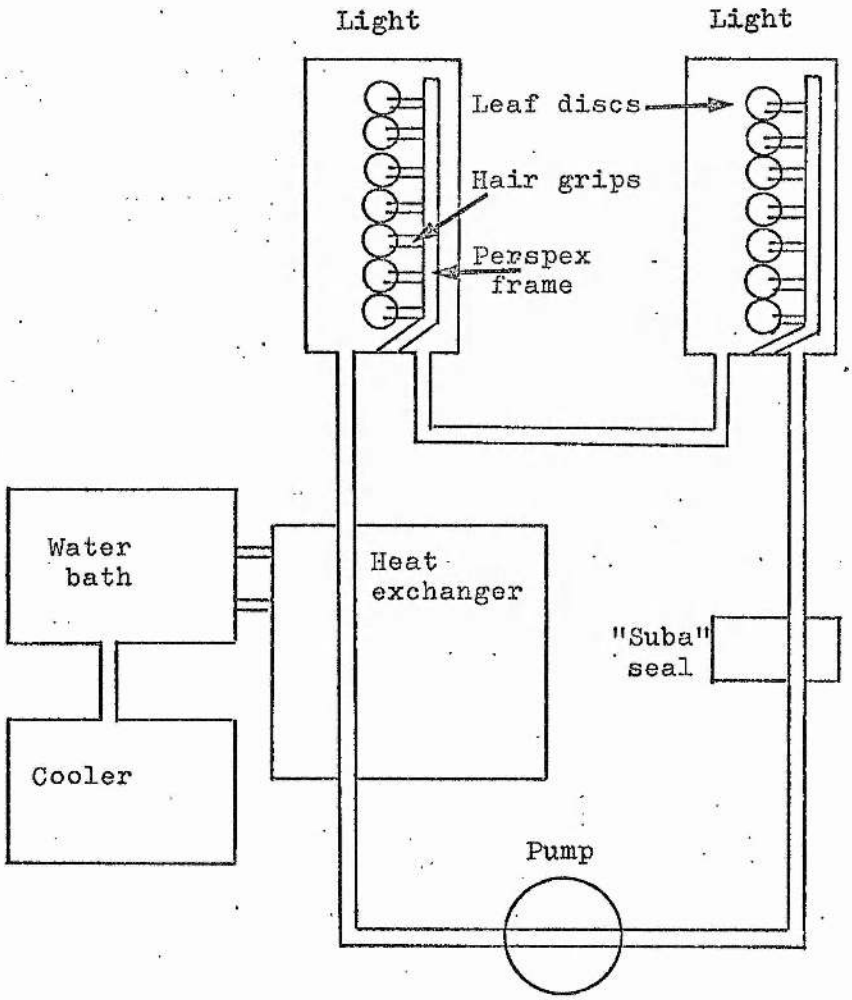


Figure 4.17

The experimental apparatus used to determine the effect of light intensity upon the incorportion of ^{14}C by cut discs of P. praelongus. The two 1.8 l kilner jar enclosures were identical and were arranged side by side with all the leaf discs in a plane normal to the direction of incident light.

The system was pumped full of incubating fluid and all the air bubbles removed. The 'suba' seal was used to inject 50 μCi of Na $H^{14}CO_3$ and the pump switched on to mix the fluid thoroughly for 2 minutes in the dark. To start the incubation the lights were switched on and two cm^3 aliquots of the bathing solution were withdrawn and precipitated in a test tube with NaOH and saturated $Ba(OH)_2$, vacuum filtered, and dried for counting. The incubations were carried out for 30 minutes and just before the end of this period a futher two 1 cm^3 aliquots were withdrawn for precipitation. To end the incubation the lights were switched off and the leaf discs removed, washed quickly in fresh medium and stuck onto planchettes for drying, weighing, and counting.

The experiment was then repeated several times using the different light intensities provided by the filter combinations L_1 - L_7 (Table 4.16) and also in the dark. The temperature of all the experiments was maintained at 15^{o}C with the heat exchanger, constant temperature water bath, and cooler (Figure 4.17). This was the temperature of the loch water at the time of collection and all the experiments were performed on the same day as collection.

Results The ^{14}C incorporation was calculated as moles x 10^{-7} CO_2 per hour, both per mg leaf dry weight and per cm^2 leaf area (Table 4.18). The standard errors of the means of the replicate discs used at each light

Table 4.18

The effect of light intensity on the incorporation of ^{14}C as moles x 10^{-7} of CO_2 per hour, on a mg leaf dry weight basis and also on a cm^2 leaf area basis, by 2.0 cm diameter discs of P. praelongus. The results are given as the means of 14 replicate discs, along with the standard error of the means.

Irradiance μ einsteins $m^{-2}sec^{-1}$	^{14}C incorporation moles x 10^{-7} h^{-1} a/ per cm^2 leaf area.	b/ per mg dry wt.
360	2.9 ± 0.37	1.142 ± 0.146
160	2.39 ± 0.36	1.193 ± 0.181
103	2.65 ± 0.47	1.213 ± 0.214
42	2.05 ± 0.36	0.953 ± 0.162
9.3	1.68 ± 0.28	0.749 ± 0.12
5.1	1.13 ± 0.22	0.602 ± 0.118
2.85	0.818 ± 0.11	0.386 ± 0.050
Dark	0.138 ± 0.05	0.063 ± 0.021

treatment are also given. The results are plotted, on a mg leaf dry weight basis, against the light intensity in microeinsteins $m^{-2}sec^{-1}$ in Figure 4.19. This shows that at low irradiances of 0-10 microeinsteins $m^{-2}sec^{-1}$ there is a marked increase of ^{14}C incorporation with increase in irradiance. This rate of increase falls off slowly until approximately 100 microeinsteins $m^{-2}sec^{-1}$ after which there is no further increase. There is, however, a suggestion of slight decrease after this up to 360 microeinsteins $m^{-2}sec^{-1}$ but the errors involved prevent any firm confirmation of this.

Discussion The results obtained above indicate that photosynthesis in a bicarbonate solution by greenhouse grown plants is easily light saturated in the laboratory at an irradiance of 100 microeinsteins $m^{-2}s^{-1}$. Therefore, the following series of flow experiments were conducted with irradiances in excess of this so that light would not be a limiting factor.

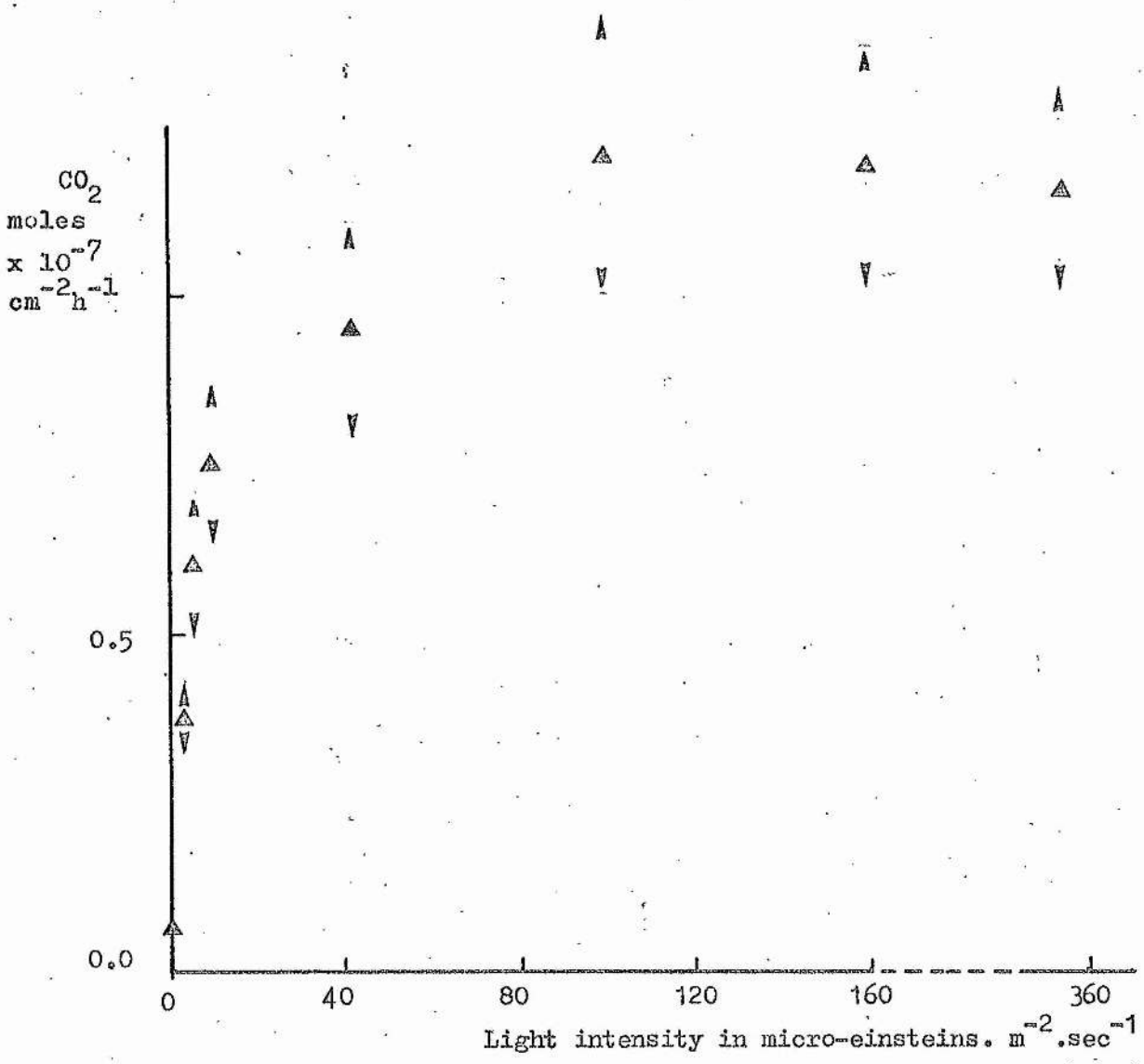


Figure 4.19

The effect of light intensity on the incorporation of ^{14}C as moles x 10^{-7} CO_2 per cm^2 h^{-1}, by 2.0 cm diameter discs of P. praelongus. The error bars show the standard error of the mean of 14 replicate discs.

4.4 The Effect of Turbulent Flow of Incubating Medium Through Experimental Enclosures on the Measured Incorporation of ^{14}C in the Light and Dark

Introduction This series of experiments investigates the effect of pumping incubating fluid around experimental enclosures, using the size of enclosures recommended in Chapter 3. The aim is to measure the extent to which ^{14}C incorporation in an enclosure is altered by turbulent flow and hence to assess the validity of productivity estimates made from enclosure experiments.

Experimental Shoots of P. perfoliatus were collected fresh from the greenhouse, where they were grown in artificial ponds, and washed in the incubating medium of 2×10^{-2} M $KHCO_3$ + 1×10^{-4} M $CaCl_2$. A shoot was placed in each of the 450 cm^3 Kilner jars, fitted with entry and exit ports attached to the lid, used as enclosures (Figure 4.20). One jar was covered in black plastic tape to act as a dark enclosure. The shoots were attached with an elastic band, by the base, to the inlet port of each jar. The jars were connected in series to a peristaltic pump and the circuit completed through a heat exchanger to maintain a constant temperature of 15°C.

The jars were sealed full of the incubating medium and all air bubbles removed. 100 μ Ci of $NaH^{14}CO_3$ were injected into the system through the 'suba' seal and the pump switched on at full speed. The fluid was then allowed to mix thoroughly for a previously

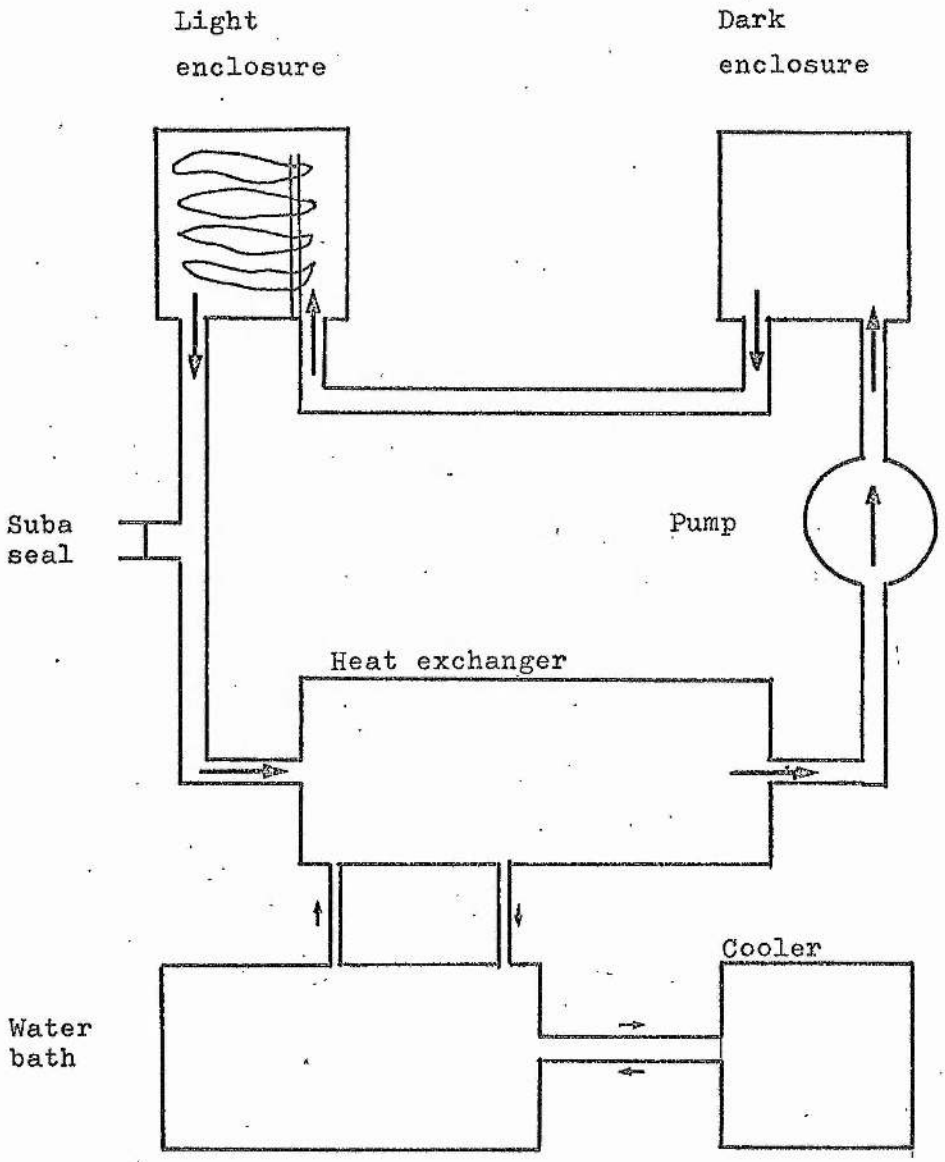


Figure 4.20

The experimental apparatus used to investigate the effect of turbulent flow on the measured ^{14}C incorporation, in detached leaves and leaves on shoots, of P. perfoliatus. The detached leaves were held in place between two glass rods (shown in the light enclosure) but the whole shoots were attached by the base to the inlet port. The direction of flow is indicated by the arrows (⟶)

determined period. All the operations were carried out in subdued lighting except for the mixing period when the apparatus was enclosed in the dark.

At the end of the mixing period, the incubation was started by switching on the overhead lights, giving 360 μ $Em^{-2}s^{-1}$. A 1 cm^3 sample of the mixed incubating fluid was withdrawn and 0·2 cm^3 aliquots of this were precipitated with 0·2 cm^3 of saturated Barium hydroxide solution, using 2·1 cm diameter glass fibre filter paper to contain the precipitate in a defined are. After 1 hour incubation a further 1 cm^3 sample of incubating fluid was removed for determination of specific activity, the shoots were then removed and washed several times in distilled water. As many as possible 6·5 mm diameter discs were cut out of each leaf and all the discs from each leaf were stuck onto a single planchette before processing for counting.

The second part of the experiment repeated the same procedure except that after the mixing period the pump was switched off for the duration of the incubating period. Thus, the two parts of the experiment only differed with respect to the movement of fluid around the shoots in the bottles.

The whole experiment was then repeated using excised leaves instead of whole shoots. These were held in a row between two glass rods, tied together with elastic bands, so that all the leaf surfaces were

at right angles to the direction of illumination.

Results The count per minute, minus background, for each planchette was corrected for self-absorption. The replicate tissues in each treatment were then averaged and the standard errors of the means calculated. The specific activity of the incubating medium was calculated as the average of the initial and final samples and was used to express the means for each treatment as moles x 10^{-7} of carbon dioxide per hour per cm^2 in Table 4.21.

This table shows that the rate of incorporation of carbon in the dark is always less than 5% of the corresponding incorporation in the light. It further shows that flow increases the incorporation of ^{14}C in the dark and in the light for both whole shoots and detached leaves by 67% and 44% respectively.

The rates of photosynthesis for the flow and static treatments are calculated by subtracting the incorporation in the dark from the incorporation in the light and expressing the result as moles x 10^{-7} of CO_2 per hour per cm^2 leaf area (Table 4.22). This shows that for both leaves on shoots and detached leaves the increase in photosynthesis in the flow treatments over the static treatments is between 42-45%. The difference in photosynthesis between detached and non-detached leaves is probably not due to the effect of detached versus non-detached *per se* but is more likely caused

Table 4.21

The measured $^{14}CO_2$ incorporation in leaves on shoots (a.) and detached leaves (b.), in the light and dark, for the static and flow treatments. Each result is given as moles x 10^{-7} CO_2 per hour per cm^2. leaf area, with the standard error of the mean for the replicate leaves or discs used in that treatment.

The figures in parentheses give the percentage increase of incorporation by the flow treatments over the corresponding static treatments.

a. Leaves on shoots.

	Light	Dark
Flow	5.28 ± 0.26	0.15 ± 0.007
	(42%)	(67%)
Static	3.72 ± 0.18	0.09 ± 0.016

b. Detached leaves.

	Light	Dark
Flow	7.88 ± 0.42	0.35 ± 0.035
	(46%)	(67%)
Static	5.40 ± 0.24	0.21 ± 0.017

Table 4.22

The rates of photosynthesis, of leaves on shoots and detached leaves, of P. perfoliatus under static and flow treatments. The photsynthesis is calculated as the difference, in moles x 10^{-7} of CO_2 per hour, between the incorporation in the light and the dark. (see table 4.21)

	Static	Flow
a. Leaves on shoots	3.61	5.13
b. Detached leaves	5.19	7.53

by the geometry of the leaves with respect to light. The detached leaves were all orientated at right angles to the direction of incident illumination and this would give higher rates of photosynthesis per unit leaf area than the whole shoot situation where the orientation is more random.

Discussion This series of experiments has shown that in experimental enclosures the effect of pumping the medium around rather than leaving it static causes a significant change in the measured ^{14}C incorporation rates for both detached and non-detached leaves.

However, although this will have a significant effect on the interpretation of in situ productivity estimates, this type of flow experiment (Westlake, 1967) does not allow calculations on the change in the diffusive resistance of the boundary layer as the flow is turbulent, non-directional, and will be complex rather than laminar. Further, the leaves do not have any defined geometry in relation to flow. This situation represents the extreme opposite to using small 25 cm^3 Mcartney bottles, with static fluid inside, for field experiments. The natural situation for a broad leaved pondweed is probably somewhere between these two extremes.

To investigate the effect of laminar boundary layers on the rate of ^{14}C incorporation two major changes in the experimental approach were adopted. First, the leaf size, shape, and orientation were

precisely defined, and secondly, a laminar flow chamber was constructed. The following series of laminar flow experiments was then conducted, incorporating both these modifications.

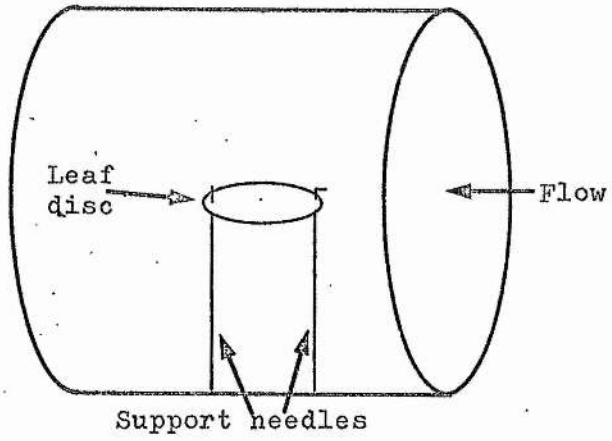
4.5 The Effect of the Laminar Flow of Incubating Medium over Discs Cut from Leaves of P. praelongus on the Measured Incorporation of $^{14}CO_2$ in the Light and Dark

Experimental Leaves of P. praelongus were collected from an artificial pond in the greenhouse and washed in the incubating medium. Discs of 2 cm diameter were cut from the middle of the leaves and they were attached to the hooked pins in the leaf disc support (Figure 4.23a). This was placed, with the laminar flow producer (Figure 4.23b) and the spacers, in the apparatus in Figure 4.24. The system was filled with incubating fluid and sealed after excluding all air bubbles present. One cm^3 of 25 μ Ci/cm^3 of $NaH^{14}CO_3$ isotope solution was added via the 'suba' seal and mixed very quickly around the system. The lights were then switched on to start the incubation period and the temperature was maintained at 15°C by means of the heat exchanger system.

Two minutes after the start, four aliquots of the incubating medium were removed and precipitated with 1 cm^3 saturated $Ba(OH)_2$ in a test tube. These precipitates were vacuum filtered and washed with hot distilled water.

After one hour the pump was stopped and the leaf disc removed, washed in distilled water, and stuck to a planchette. This procedure was repeated several times

a/ Leaf disc support



b/ Laminar flow producer

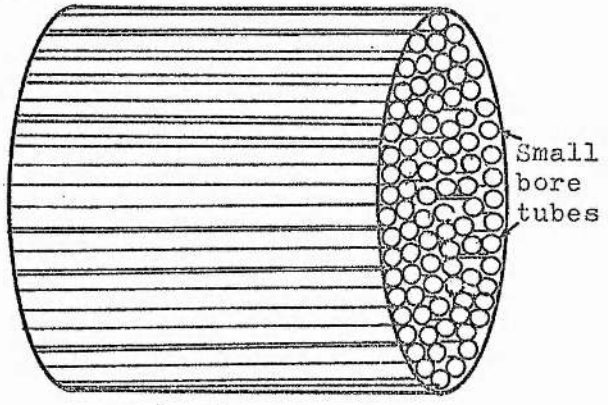


Figure 4.23

The leaf disc support (a) and the laminar flow producer (b) which form part of the assembly in figure

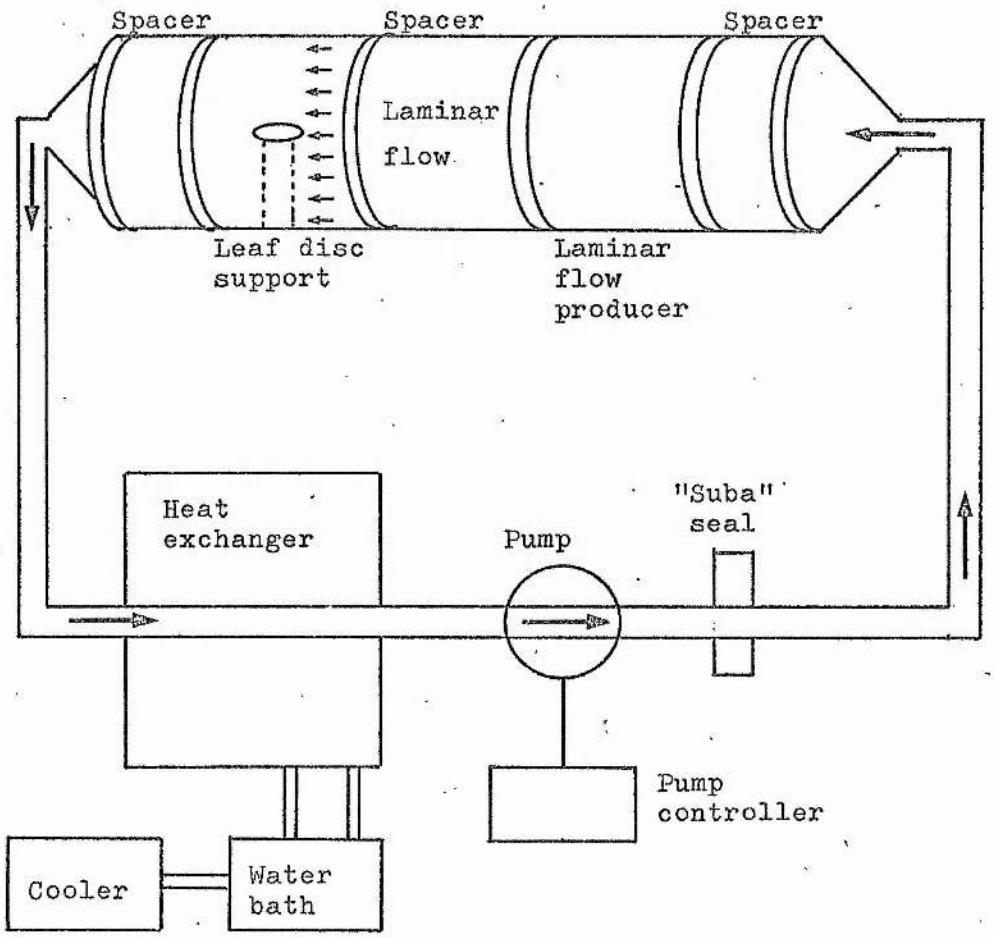


Figure 4.24

The apparatus used to produce a laminar flow of incubating fluid over the surface of discs cut from leaves of P. praelongus. The leaf disc support, laminar flow producer, and the three spacers are clamped inside a perspex tube using "o" rings between them to form a seal.

at two different laminar velocities in the light and also in the dark. Both the tissues and the precipitates were dried before counting and correction for self-absorption.

The whole experiment was repeated with the following alterations. The original bathing solution of 2×10^{-2} M $KHCO_3$ was replaced by 2×10^{-3} M $KHCO_3$, and 0·2 cm^3 rather than 1 cm^3 aliquots of this were taken for precipitation. More replicate leaf discs under each of the flow conditions were used.

The peristaltic pump used was controlled electronically to maintain a constant speed irrespective of load and could be adjusted to obtain a particular flow rate. For this experiment, two settings were chosen which gave laminar flow velocities, at the leaf disc, of 10 and 60 mm/sec. respectively. These were measured by injecting ink and timing the movement along a known distance of the tube at different pump control settings.

The illumination for these experiments was provided by the overhead light system (Figure 4.7) fitted with neutral density filters giving a light intensity of 300 μ einsteins $m^{-2}sec^{-1}$.

Results The ^{14}C incorporation rates as moles $\times 10^{-7} CO_2$ per hour for each disc used in the experiments, are given in Tables 4.25 and 4.26. The means and standard deviations of the means of the treatments in each experiment are given in Table 4.27 and 4.28, along

Table 4.25

The ^{14}C incorporation rates of 2.0 cm diameter leaf discs of P. praelongus, in 2×10^{-2} M $KHCO_3$, at the two laminar flow velocities of 60 and 10 mm per second. They are expressed moles x 10^{-7} CO_2 per hour both on a leaf area basis and on a leaf dry weight basis.

Leaf disc	Flow mm/sec.	Light intensity	Incorporation M x 10^{-7} CO_2 per cm^2	per mg
1	60	Light	5.97	5.07
2	10	"	1.87	1.86
3	10	"	4.09	3.90
4	60	"	2.81	2.76
5	60	Dark	0.21	0.16
6	60	Light	7.84	6.01
7	10	"	5.59	4.18

Table 4.26

The ^{14}C incorporation rates, in the light, of 2.0 cm diameter leaf discs of P. praelongus in 2 x 10^{-3} M $KHCO_3$ at the two laminar flow velocities of 60 and 10 mm per second. The rates are expressed as moles x 10^{-7} CO_2 per hour per cm^2 leaf area.

Disc no.	Flow 60 mm/sec	Disc no.	Flow 10 mm/sec
1	3.94	3	3.32
2	4.07	4	3.53
5	4.68	6	3.76
7	4.31	8	2.31
9	2.36	10	3.41
11	6.2	12	3.74
13	3.96	14	3.32
15	5.24		

Table 4.27

The comparison of the means of the rates of ^{14}C incorporation, of the two samples of leaf discs in table 4.30 , measured at laminar flow velocities of 60 and 10 mm/sec.

	Laminar flow velocity mm/sec.	
	60	10
Number of observations	3	3
Average ^{14}C incorporation in the light as moles x 10^{-7} /cm^2/hour	5.54	3.85
Standard deviation of the samples	2.542	1.872
Standard deviation of the means	1.468	1.081
Standard deviation of the difference between the means	1.823	
Difference of the means	1.69	
t value for 4 degrees of freedom	0.927	

Table 4.28

The comparison of the means of the rates of ^{14}C incorporation, of the two samples of leaf discs in table 4.31, measured at lamin-ar flow velocities of 60 and 10 mm/sec.

	Laminar flow velocity mm/sec.	
	60	10
Number of observations	8	7
Average ^{14}C incorporation in the light as moles x 10^{-7} /cm^2/hour	4.345	3.3414
Standard deviation of the samples	1.114	0.4897
Standard deviation of the means	0.394	0.1851
Standard deviation of the difference between the means	0.4352	
Difference of the means	1.004	
t value for 13 degrees of freedom	2.3061	

with the 't' value for comparison, of the ^{14}C incorporation at the two laminar flow velocities.

The effect of different laminar flow velocities on the ^{14}C incorporation in 2×10^{-2} M $KHCO_3$ was examined by comparing the means of the three replicate discs at each velocity (Table 4.27). This shows that the standard deviation of each sample is high and this is reflected in a high standard deviation of the difference between the means. A null hypothesis, that there is no difference between the means, was tested by comparing the calculated 't' value with the maximum expected from a normally distributed population for four degrees of freedom (Table 4.27). The permissible 't' value of 2·78, at the 95% significance level, is three times the calculated 't' value of 0·927 and indicates that there is a 95% probability that there is no difference between the means.

The same analysis was applied to the comparison of the ^{14}C incorporation at two laminar flow velocities in 2×10^{-3}M $KHCO_3$. In this case, the calculated 't' value (2·306) was greater than the maximum permitted (2·16) for 13 degrees of freedom at 95% significance level (Table 4.28). This would indicate a significant difference between the means. However, the standard deviations of the samples (1·114 and 0·490) are very different and the results of the simple 't' test must be accepted with caution.

A variance ratio was, therefore, calculated and compared to the maximum expected variance ratio, for a normal distribution at 95% probability, assuming that both samples were drawn from a single population. The calculated value (5·18) exceeds the expected value (4·25) and indicates that the two samples have come from different populations.

The rates of ^{14}C incorporation at the two laminar velocities 60 and 10 mm. per sec., are different both in 2×10^{-2}M $KHCO_3$ and 2×10^{-3}M $KHCO_3$ but the lack of significance in the 2×20^{-2}M $KHCO_3$ will be due to the smaller number of replicates used and the high variability of incorporation rates by discs of P. praelongus. Discussion This means, therefore, that changing the rate of laminar flow over a cut leaf disc produces measurable changes in the rate of ^{14}C incorporation. Using the previous analysis of laminar boundary layers a diffusion analysis can be applied to the discs at the two flow velocities.

Cut leaf discs were chosen to avoid the problems inherent with using leaves of different sizes and shapes. A problem with using any shape is in deciding what value to give to the laminar boundary layer thickness as this is a function of the distance from the leading edge of the surface. A reasonable solution to this problem would be to consider the concept of an average boundary layer thickness. This can be calculated by dividing the

volume of the laminar boundary layer, over the whole surface, by the area of the surface.

The volume (v) of the laminar boundary layer over the surface of a disc will be given by (Appendix III)

$$v = 10\, V^{\frac{1}{2}} U^{-\frac{1}{2}} \int_{0}^{2r} L^{\frac{1}{2}} \mathrm{Tan}^{-1} \left[2L^{-1} \left(r^2 - \frac{L^2}{4} \right) \right]^{\frac{1}{2}}$$

Solutions of this integral function have been obtained using Simpsons Rule for the two laminar flow velocities ($U_1 = 0{\cdot}01$ m s^{-1} and $U_6 = 0{\cdot}06$ m s^{-1}) to give $v_1 = 1{\cdot}64 \times 10^{-6} m^3$ and $v_6 = 6{\cdot}68 \times 10^{-7} m^3$. The area of the disc is $3{\cdot}142 \times 10^{-3}$ m^2 and dividing this into v_1 and v_6 gives $d_{av} = 5{\cdot}21 \times 10^{-3}$ m at $0{\cdot}01$ m s^{-1} and $d_{av} = 2{\cdot}13 \times 10^{-3}$ m at $0{\cdot}06$ m s^{-1}.

These values can be used in the following diffusion equation across the laminar boundary layer;

$$\text{Flux of } {}^{14}C = D \times \frac{C_{bulk} - C_{leaf}}{d_{av}}$$

where D = the diffusivity, C_{bulk} and C_{leaf} = the concentrations of carbon in the bulk solution and at the leaf surface respectively. The value of the ^{14}C flux used in this equation will be half the measured value as this expressed as ^{14}C incorporation for one side of the disc only. Substituting this and rearranging for C_{leaf} gives the following expression;

$$C_{leaf} = C_{bulk} - \frac{\frac{flux}{2} \times d_{av}}{D}$$

Solving this equation using C_{bulk} as equal to the concentration of inorganic carbon in the incubating medium (2×10^{-3} M) and D = $1{\cdot}42 \times 10^{-5}$ (for CO_2 in water at 15°C) gives values for C_{leaf} of $4{\cdot}25 \times 10^{-4}$ M and $8{\cdot}0 \times 10^{-4}$ M at flow velocities of 0·01 m s^{-1} and 0·06 m s^{-1} respectively. However, solving this equation using C_{bulk} as equal only to the concentration of free carbon dioxide in the incubating medium ($2{\cdot}16 \times 10^{-5}$ M free carbon dioxide) becomes impossible as;

$$\frac{\frac{flux}{2} \times d_{av}}{D} > C_{bulk}$$

Hence diffusion of free carbon dioxide alone, across the theoretical laminar boundary layer, would not be fast enough to support the measured fluxes of ^{14}C. This situation would not occur as the bicarbonate present would act as a supply of free carbon dioxide and the next sections of this chapter consider the rate at which this would happen.

4.6 Uncatalysed Dehydration of Bicarbonate

The significant difference between carbon dioxide concentrations in aquatic and terrestrial systems has been shown to be the concentration of free carbon dioxide in equilibrium with bicarbonate and its dependance on the value of pH. Removal of free carbon dioxide in water will not reduce the concentration of free carbon dioxide in direct proportion to the amount removed. The pCO_2 buffering capacity will be significant and due to dehydration of the bicarbonate ion according to equations 2, 3 and 5 (Chapter 3 section 2).

$$CO_2 + OH^- \underset{k_{HCO_3}}{\overset{k_{OH}}{\rightleftharpoons}} HCO_3^- \qquad (5)$$

$$H_2CO_3 \underset{}{\overset{KH_2CO_3}{\rightleftharpoons}} HCO_3^- + H^+ \qquad (3)$$

$$CO_2 + H_2O \underset{k_{CO_2}}{\overset{k_{H_2CO_3}}{\rightleftharpoons}} H_2CO_3 \qquad (2)$$

Equations 2, and 3, can be rewritten as

$$CO_2 + H_2O \underset{k_d}{\overset{k_{CO_2}}{\rightleftharpoons}} HCO_3^- + H^+ \qquad (2.3)$$

and the rate equation for (5) and (2.3) may be written as follows (Magid and Turbeck, 1968)

$$\frac{-d\left[HCO_3^-\right]}{dt} = k_d\left[H^+\right]\left[HCO_3\right] + k_{HCO_3}\left[HCO_3^-\right] - k_{CO_2}\left[CO_2\right] - k_{OH}\left[OH^-\right]\left[CO_2\right]$$

which can be simplified to (Magid, 1967)

$$\frac{-d\left[HCO_3^-\right]}{dt} = k'_d \left[HCO_3^-\right] - k'_h \left[CO_2\right]$$

in which $k'_d = k_d\left[H^+\right] + k_{HCO_3}$ and $k'_h = k_{CO_2} + k_{OH}\left[OH^-\right]$

Values for k'_d and k'_h and their dependence on pH and temperature have been determined experimentally by Magid and Turbeck (1968), and are given in Table 4.29. This shows that the value of k'_d varies by an order of magnitude between 0-25°C and by almost as much between pH 7-8. The kinetics of hydration (k'_h) show an even greater variation with temperature but is less affected by pH between 7-8.

Using this equation, it is possible to calculate the maximum uncatalysed rates of formation of carbon dioxide from bicarbonate ions (Everson, 1970). When the carbonic acid system is in equilibrium then;

$$k'_d \left[HCO_3^-\right] = k'_h \left[CO_2\right]$$

and $\frac{-d\left[HCO_3^-\right]}{dt}$ will be zero. Removal of free carbon will cause $k'_h \left[CO_2\right]$ to tend towards zero and at the limit;

$$\frac{-d\left[HCO_3\right]}{dt} = k'_d \left[HCO_3^-\right]$$

This gives the maximum rate of dehydration of bicarbonate possible. For a bicarbonate solution of $\left[HCO_3^-\right] = 2 \times 10^{-3}$ M and using an estimated value of $k'_d = 0{\cdot}4 \times 10^{-3}\ s^-$

Table 4.29

The variation of the dehydration coefficient of bicarbonate (k'd) and the hydration coefficient of carbon dioxide (k'h), with temperature and pH (Magid and Turbeck 1968).

a) At pH = 7.0

Temperature ^{o}C	k'd x $10^{-3}s^{-1}$	k'h x $10^{-3}s^{-1}$
25	7.1	47
18	3.6	23
10	1.75	9.6
0	0.72	2.3

b) At $25^{o}C$

pH	k'd x $10^{-3}s^{-1}$	k'h x $10^{-3}s^{-1}$
7.0	7.1	47
7.2	4.2	44
7.4	2.8	46
7.6	1.8	48
7.8	1.2	49
8.0	0.9	56

(at 15°C and pH 8), the value of $\frac{-d\left[HCO_3^-\right]}{dt}$ will equal $0{\cdot}8 \times 10^{-6}$ moles $l^{-1}s^{-1}$.

Several problems with using this value in any diffusion model will exist. The strong dependence of k'_d on pH means that the pH increase caused by the removal of free carbon dioxide by the photosynthesising leaf surface will reduce the rate of dehydration. This will be in particular the case inside the boundary layer, nearest to the leaf surface, where the CO_2 will be lowest and hence $k'_h\left[CO_2\right]$ will also be lowest.

However, it will also be nearest to the leaf surface that any released organic material will be greatest. The presence of any exogenous carbonic anhydrase here will dramatically affect any diffusion model and the properties of this catalyst of the dehydration of bicarbonate are considered next.

4.7 Catalysed Dehydration of Bicarbonate

Carbonic anhydrase, the carbon dioxide catalyst in blood, was first discovered by Meldrum and Roughton (1932). It was first observed in plants by Neish (1939) and has a widespread activity in the leaves of flowering plants (Bradfield, 1947; Waygood and Clendenning, 1950; Kondo, Yonezawa and Chibah, 1952; Everson, 1969; Kisiel and Graf, 1972). It is also found in marine algae (Bowes, 1968), fresh water algae (Graham and Reed, 1971) and fresh water higher plants (Steeman-Nielsen and Kristiansen, 1949). It is described as a powerful bio-catalyst of the reversible process of CO_2 hydration (Struoginite, 1972), its rate of catalysis being amongst the fastest known and pH dependent (Koenig and Brown, 1972). It is a metaloenzyme, containing twelve atoms of zinc per protein with a molecular weight of about 30,000 (Koenig and Brown, 1972). It catalyses reaction (2),

$$CO_2 + H_2O \underset{kH_2CO_3}{\overset{kCO_2}{\rightleftharpoons}} H_2CO_3 \qquad (2)$$

which is slow compared to reaction (3)

$$H_2CO_3 \overset{kH_2CO_3}{\rightleftharpoons} H^+ + HCO_3^- \qquad (3)$$

and the catalysis by carbonic anhydrase may be considered as acting on the following equation (2.3)

$$CO_2 + H_2O \underset{k_d}{\overset{kCO_2}{\rightleftharpoons}} HCO_3^- + H^+ \qquad (2.3)$$

This reaction (2.3) occurs in the absence of enzyme but it has been suggested that this does not happen fast enough for normal metabolism of cells and organisms (Bowes, 1968; Gibons and Edsall, 1964). There is no evidence for the other interconversion of carbon dioxide and bicarbonate (5),

$$CO_2 + OH^- \rightleftharpoons HCO_3^- \qquad (5)$$

being catalysed by carbonic anhydrase (Carter, 1972).

Carbonic anhydrase is of fundamental importance when carbon dioxide is transported or exchanged (Bowes, 1968), and is implicated in carbon dioxide fixation (Everson and Slack, 1968; Chen, Brown and Black, 1970). It is found in both monocots and dicots, although they have different characteristics. A monocot type and a dicot type of the enzyme have been observed (Atkins, Patterson and Graham, 1972(a)). The monocot type is more labile in solution (Atkins, Patterson and Graham, 1972(b)). It has been suggested that there was a significant difference in localisation and level of activity of the enzyme in C_3 and C_4 plants (Chen, Brown, and Black, 1970; Everson, 1971; Everson and Slack, 1968; Graham, Atkins, Reed, Patterson, and Smillie, 1971) and that this was related to the different proposed substrates for the two carboxylating enzymes. Carbon dioxide for ribulose diphosphate carboxylate (Cooper, Filmer, Wishmick, and Lane, 1969) and bicarbonate for phosphoenolprymate carboxylase (Maruyama,

Easterday, Chang and Lane, 1966). These two enzymes have now been shown to both use carbon dioxide (Waygood, Mache and Tan, 1969; Cooper, Tchen, Wood and Benedict, 1968) and the low level of activity of carbonic anhydrase in C_4 plants has been shown to be caused by problems of measurement (Poincelot, 1972).

In C_3 plants an increase in the carbon dioxide concentration causes a reduction, while a decrease in the carbon dioxide concentration causes an increase, in the enzyme activity (Cervigni, Teofani, and Bassanelli, 1971). The converse is found for C_4 plants. It has been suggested that the chemical resistance in photosynthesis bears a relationship to the amount of carbonic anhydrase in the leaf (Dowton and Slayter, 1972). Enns (1967) demonstrated that carbonic anhydrase will enhance the rate of transport of carbon dioxide across a membrane by as much as one hundred fold. Findenegg (1973) found that Scenedesmus adapted to low and high carbon dioxide levels, with high and low levels respectively of carbonic anhydrase. At a low pH (5·8) there was not much difference in photosynthesis between the two adaptations but at a high pH (9·2) the high carbon dioxide adapted cells did not photosynthesise much compared to low carbon dioxide adapted cells. This is suggested as implicating carbonic anhydrase in the uptake of bicarbonate.

The physiological functions of carbonic anyhdrase have been explained by several suggested methods of action (Poincelot, 1971; Zelitch, 1971). Waygood et al (1969) suggest that the role of carbonic anyhdrase in green plants is to trap carbon dioxide that may escape into the atmosphere vio glycolate oxidase in photo-respiration. Werden and Heldt (1972) showed that bi-carbonate accumulated in the stroma of isolated spinach chloroplasts and that the main carbonic anhydrase activity was located in the chloroplast. Inhibition of carbonic anhydrase inhibited the rate of bicarbonate uptake and they proposed that carbon dioxide diffusion, facilitated by carbonic anhydrase, across the inner membrane was rate controlling. Graham and Reed (1971) also proposed control of photosynthesis by carbonic anhydrase overcoming a permeability barrier for carbon dioxide at the chloroplast membrane. Tobin (1970) and Zelitch (1971) discuss the role of carbonic anyhdrase as catalysing the conversion of the storage form (bicarbonate) to the active form (carbon dioxide) of the substrate for the carboxylating enzymes, phosphoenol pryurate and ribulose diphosphate.

The significance of carbonic anhydrase in the transport of carbon dioxide across membranes, its ability to supply carbon dioxide from a pool of bicarbonate, and its implications in the control of photosynthesis in relation to the external concentrations of carbon dioxide, indicate its possible involvement in photosynthesis of aquatic higher plants.

However, there would be a significant difference in the action of the enzyme in aquatic plants compared to terrestrial plants if it was able to be 'leaked' out of cells to the intercellular spaces and then into the layers of water immediately adjoining the leaf surface. Here in the boundary layer it would have a dramatic effect on the kinetics of the dehydration of bicarbonate and as such provide a potential source of carbon dioxide.

CHAPTER FIVE

DISCUSSION

5.1 Introduction

To measure the rates of photosynthesis of terrestrial plants it has been necessary to control and simulate environmental factors surrounding the plant. This incurs problems since we are concerned with; controlling the spectral composition of the incident light, measurement and control of the heat load on the plant, control of the movement of air over the plant, and the water relations of the plant. These and other problems have usually led to the development of small chambers in which a single leaf can be enclosed under the most precisely defined conditions (Woolhouse, 1967).

In situ methods have been developed in order to measure rates of photosynthesis under natural environmental factors. Westlake (1973) reviewed the environmental factors affecting the growth of aquatic macrophytes in streams and included; water chemistry, water velocity, bottom deposits, temperature, light, and turbidity. He also observed that as the flow rate decreased the plant populations came closer to those of lakes and ponds.

Enclosure of leaves in small chambers can cause different problems in aquatic and terrestrial situations. Control of air movement is much easier to

organise than control of water movement in a submerged chamber and this is not usually achieved. Water chemistry, particularly that of carbonic acid, is a factor unique to aquatic *in situ* experiments. Similarly, water relations is a factor unique to terrestrial leaves.

However, in both cases the heating effect of the light must be considered. This is a problem in the terrestrial situation where overheating of leaves can be a naturally occurring problem and small chambers would exacerbate this by preventing further heat loss. In ponds, although the water temperatures have been found to be very variable in the summer and mainly influenced by the amount of sunshine received (Martin, 1972), the absorption of light by an aquatic leaf is less likely to induce overheating for two reasons. Firstly, the underwater irradiance is lower than in air and secondly, water has a higher specific heat and will require more energy to produce the same temperature rise as in air. Therefore, it is unlikely that heating of leaves will be a problem in underwater *in situ* enclosures.

This discussion is concerned with environmental factors that may be altered by the procedures involved in an *in situ* field experiment and the degree to which these changes can be kept to a minimum or compensated for. Problems in the techniques of the ^{14}C method are also considered.

5.2 The Problems Encountered with the use of the ^{14}C Technique and the Recommendations Arising from this Work for Overcoming them

In this study certain problems have been encountered with regard to the ^{14}C *in situ* field technique. A detailed study of these in Chapter 2 has resolved several of these problems and recommendations can be made for further field productivity studies of aquatic macrophytes.

The ^{14}C incorporation by a whole leaf correlates with the position of the leaf on its shoot (Figure 2.3), and the selection of leaves, for obtaining meaningful replication in different experimental regimes, will be important. It must be realised that if submerged aquatic plants are collected by means of a remote grab then information about the growing position will be lost and further the leaves, if brought to the surface, will suffer exposure and handling. Therefore, it is essential for the plant material to be collected first hand and placed in enclosures at the required depth, by aqualung diving. A diver has poor control over his depth and station, and will therefore tend to collect leaves from a depth range of several feet. This error in depth of collection will be important as the underwater environment (i.e. light intensity) changes more rapidly over a depth of a few feet than does a comparable terrestrial situation. This means that leaves will have to be

selected carefully and many replicates used if it is intended to extrapolate from single leaves to whole populations.

If discs are cut from leaves prior to incubation then the position of the disc on the leaf is important in determining the rate of photosynthesis (Figure 2.4 and Table 2.7). The results in Table 2.6 suggest that the ^{14}C incorporation is more likely to correlate with the position of the disc on the leaf than the size of the disc itself.

The procedure for counting the ^{14}C activity of both the tissues and the precipitates (2.7) was devised to be of practical use in the field. This study shows that the different geometry present in these two types of samples does not allow the samples to be counted with the same efficiency. Self absorption corrections have to be made and these do not apply equally to both the tissues and the precipitates. Therefore, for accurate work it would be best to count the samples in the liquid or gas phase. Liquid scintillation counting is a technique that could be applied but would require careful processing of the ^{14}C incubating media in the field to prevent gaseous loss.

To retain ^{14}C counting in the solid phase would necessitate accurate calibration of the counting procedure by determining the absolute activity of a representative number of samples to produce a valid self absorption correction for each type of sample used

(Light, Ellis-Evans and Priddle, 1981).

A remaining problem encountered with the ^{14}C field technique is concerned with interpreting the measured ^{14}C incorporation rates as either net or gross photosynthesis. The results of experiments to measure the rate of release of previously fixed carbon (Chapter 2.7) show that this is greater in the dark than in the light. This apparent absence of photorespiration, at high light intensity and high oxygen concentration, is not what would be expected for a normal Calvin cycle plant. During photosynthesis in aquatic plants there will be a build up of the oxygen concentration surrounding the leaf, and this would be expected to further promote photorespiration. It would appear that photorespiration is occurring but the released carbon is being refixed before it can escape to the bulk solution. Similar conclusions have been drawn for other submerged hydrophytes by Wetzel and Hough (1973), Stanley and Naylor (1972) and Hough (1974).

Hough (1974) suggests that as submerged hydrophytes are usually exposed to lower, maximum oxygen concentrations, light, and temperature than are terrestrial plants, the Hatch and Slack pathway may not be of major adaptive value in submerged plants. Photorespiration will affect the efficiency of photosynthesis and regardless of the extent of refixation considerable photosynthetic assimilatory reducing power is lost in

photorespiration. However, it is noteworthy that the main advantage of the Hatch and Slack system in terrestrial plants is probably as a strategy to reduce water loss and therefore adaptive comparisons must be treated with caution. Raven (1978) states that terrestrial plants can be classified into C.A.M., C_3 or C_4 from a standpoint of biochemistry and physiology but there is no such clear cut distinction in photosynthesis type when submerged aquatic plants are considered. Further, Jana and Choudhuri (1979) found that submersed aquatic plants might show characteristics of either C_3 or C_4 plants depending on their environmental conditions. They further suggest that photorespiration in aquatic plants is limited in comparison with terrestrial C_3 plants because of extensive refixation of photorespired carbon dioxide which may be related to the slow rate of diffusion of carbon dioxide from the leaves of these plants. It may also be that the photorespiratory capacity of submersed aquatic plants is less than that of terrestrial plants because of the limited solubility of oxygen in water (Van, Haller and Bowes, 1976).

The extensive refixation of carbon dioxide demonstrated in this study (Table 2.30), the lack of a clear cut distinction in photosynthetic type (Raven & Glidewell, 1978) and variability induced by the environment (Bowes, Holaday, Van and Haller, 1977) all contribute to an uncertainty with regard to the measurement of net or gross photosynthesis. It is, therfore, recommended that

this refixation of carbon dioxide be measured for samples of plant material to be used in *in situ* ^{14}C productivity estimates.

A further potential problem with photosynthesis experiments using single detached leaves was that of the supply of carbon dioxide from the substrate. Although this was shown to occur to a negligible extent in the species under study other workers have implicated this route as being a significant source of exogenous carbon. Having shown that sufficient carbon dioxide does not diffuse from the air (at Baltimore) to keep *Elodea* growing or alive, during the summer and spring months, Brown (1913) suggests that the substrate may serve as an important source of carbon dioxide. Misra (1936) also considers the release of carbon dioxide from the mud into the water and Bristow (1969) states that although levels of free carbon dioxide are usually low in lakes and streams they are considerably higher just above the mud. He further indicates that whether significant amounts of carbon dioxide can be absorbed by the roots of aquatic plants remains to be tested.

The hollow stems of herbaceous plants in moist mountain meadows and bogs contain laccunal air passages connecting the leaves with the roots. Air and gases can move from one part to another and it may be possible for the transfer of carbon dioxide from the root to the

shoot (Billings and Godfrey, 1967). They found high internal carbon dioxide concentrations ranging from twenty to fifty times the air concentration. Frank and Hodgson (1964) studied absorption and translocation in submerged plants but found no translocation of a herbicide. Hartman and Brown (1967) showed that once carbon dioxide was absorbed by the roots of submerged vascular hydrophytes it could diffuse readily from the roots to the upper parts of the plants. They considered the laccunal system to be a reservoir for a rather significant volume of gases. This volume is such that when shoots of Potamogetons are cut from their roots they float quickly to the surface (Personal observation and problems encountered by Rich, Wetzel, and Van Thuy, 1971). Denny (1981) has recently criticised the lack of experiments on whole plants under controlled conditions simulating the natural environment and that more frequently information has been obtained on whole plants under artificial conditions. However, the main criticism of the experiment performed in this study, that of proof of a leak proof seal separating the experimental solutions, does not invalidate the finding of a very low rate of translocation carbon dioxide from the root to the shoot.

However, if detached leaves are used in ^{14}C productivity studies then interpretation of these rates will depend on an understanding of the internal movement of carbon dioxide in the plants studied.

5.3 Exogenous Inorganic Carbon Environment

In contrast to terrestrial plants, aquatic plants are exposed to several different sources of carbon dioxide and hence a completely different situation exists to that outside a terrestrial leaf. It can be shown that the concentration of free carbon dioxide is pH controlled in closed systems (Chapter 3.2) and further that a whole water body may behave as a partially closed system (Chapter 3.3). Super saturated oxygen has been shown to escape to the air more easily than a carbon dioxide deficit can be restored from the limited supply of carbon dioxide in the air (Wood and Verduin, 1972). Liss (1973) shows that for sparingly soluble gases such as oxygen and carbon dioxide the flux from air to water is controlled by the liquid phase resistance. Bolin (1960) indicates that although carbon dioxide is considerably more soluble than oxygen in water it is generally assumed that the diffusion processes in the liquid predominate transport across the interface. The slow rate of hydration is also involved (Liss, 1973) and at higher pH values, where some of the carbon dioxide occurs as ionic species, there are similar concentration gradients for bicarbonate and carbonate (Hoover and Berkshire, 1969). Dankwerts (1970) proposes a film model for laminar flow close to the air/water interface. The presence of carbonic anhydrase has been shown to increase the rate of carbon dioxide in sea water by a factor of

about twenty (Berger and Libby, 1969). Therefore, whole water bodies, such as Lochs, will be expected to maintain carbon dioxide deficits compared to the atmosphere.

Although a water body may be somewhat isolated from the atmospheric supply of carbon dioxide, the buffering capacity of the bicarbonate system in the water will prevent the carbon dioxide level dropping in direct proportion to the amount removed (Chapter 3.3). Various authors indicate that above pH 8·4 (Goldman, Porcella, Middlebrooks, and Toerion; 1971) or pH 9 (Jackson, 1964) there is very little inorganic carbon present as free carbon dioxide. Lowenhaupt (1956) state that photosynthesis is blocked in non bicarbonate users when the pH value is greater than 9, i.e. when free carbon dioxide is 'negligible'.

It has been shown that in the loch waters under question the free carbon dioxide concentration is still biologically very significant as pH 9 (Chapter 3.3). The presence of bicarbonate in natural waters of the order of 10^{-3} M as opposed to the concentration of free carbon dioxide being $1\text{-}2{\cdot}5 \times 10^{-5}$ in fresh waters is stressed by Hutchinson (1967).

The problem of exhaustion or depletion of carbon in an enclosure will depend upon the ratio of photo-synthesising tissue to the volume and concentration of carbon in the incubating fluid in an enclosure. The

effect of this on the pH changes occurring during *in situ* and laboratory experiments are given in Chapter 3 section 4 and 5. These can be compared to the pH changes occurring in the loch water (Chapter 3.3);

	pH change
Laboratory	1•5
In situ	1•0
Loch water	0•2

The *in situ* pH change is similar to that occurring in the laboratory situation for small enclosures, but the natural pH changes that might be expected to occurr in the loch water during an *in situ* experiment bear no relation to these. The laboratory experiments confirm the relation between tissue amount and incubating volume for a given concentration of carbon. This points to the necessity for using large enclosures with small amounts of plant tissue for short period. This conclusion is also indicated by Drew and Robertson (1974) for *in situ* photosynthesis experiments with macrophyte marine algae using the Winkler technique for measurement of oxygen exchange. The problem will depend upon the rate of photosynthesis and the amount of available carbon. For *In situ* experiments in Lochs such as Meadie (Table 3.9) there will be little buffering capacity and the pH changes could be very large.

Olsen (1953) suggested that over a wide range of pH, the pH value of the solution itself has no direct influence on the rate of ion uptake in higher plants.

However, if the pH change represents the shift in proportion of carbon dioxide and bicarbonate (Figure 3.3), and if bicarbonate has a lower rate of fixation than carbon dioxide, then the effect of pH will affect the uptake of carbon. In general, the semi-diurnal character of pH in natural waters is related to algal growth and sunlight (Palmer and Izatt, 1970) but in the experiments conducted in Lochs Borralie and Croispol (Chapter 3.3) it is more likely to be related to macrophyte photosynthesis.

Recent experiments by Allen and Spence (1981), and their knowledge of the prevailing alkalinity values in nature lead them to conclude that it is unlikely most macrophytes assimilate a significant proportion of their carbon dioxide directly from bicarbonate, until the pH exceeds about 9 when the photosynthetic rate will be about 10% of the potential rate. They also showed the effect of pH on the photosynthesis of macrophytes to be very significant. The macrophytes reached their maximum rate around pH 7·5 and declined to about 10% maximal rate by pH 9·5.

The pH changes occurring during some laboratory and <u>in situ</u> photosynthesis experiments in this study are more than 1 pH unit greater than those measured in the lake water itself. Consequently, the rates of photosynthesis measured will be significantly affected by the pH. The experiments with different bottle sizes

and tissue weight per bottle (Figure 3.20 and 3.21) provide a recommendation for using small amounts of tissue with large enclosures for *in situ* experiments and to monitor the pH change to ensure that it does not exceed that occurring in the lake itself.

5.4 Water Movement

The supply of carbon dioxide to leaves of aquatic plants will be significantly affected by the movement of water and the enclosure of a leaf will alter this affect. Changes in water movement around an aquatic leaf will be more important than changes in air movement around a terrestrial leaf. The effect of flow on the photosynthesis of submerged plants was noted by James (1928). He also indicated that this only occurred when carbon dioxide was the only carbon source present and that the buffering capacity of the bicarbonate ion could overcome the effects of flow. Olsen (1950 and 1953) stressed the role of stirring in the absorption of nutrients, by higher plants, in water culture. He further showed that green leaves and stems of aquatic plants suffer from the same problems and this effect was interpreted as due to the presence of unstirred layers. These have been observed to affect the determination of permeability coefficients of membranes and under high water velocities have been given as in the range 12 to 48 m x 10^{-6} (Ginzburg andKatchalsky, 1963). Dainty (1963), in discussing the water relations of plants, gives the thickness of these unstirred layers as being between 20 and 500 m x 10^{-6}. He states that the actual thickness depends upon the size of the object and the rate of stirring. He considers them not as an unstirred layer but rather as a region of slow laminar flow parallel to the membrane surface. In their work

on unstirred layers in frog skin Dainty and House (1966) found thicknesses of between 40 and 170 m x 10^{-6} depending on the rate of stirring. They also observed different thicknesses occurring on the inside and the outside of the skin, suggesting an effect due to different surfaces. The dependence of measured unstirred layers on the size of the object is demonstrated by the thickness of unstirred layers around red blood cells as $5{\cdot}5 \pm 0{\cdot}8$ m x 10^{-6} (Sh'afi, Rich, Sidel, Bossert, and Solomon; 1967). Polle and Jenny (1971) conclude that boundary layer effects in ion absorption by roots and storage organs of plants are more significant when concentrations are low. They further discuss the presence of a laminar sub-layer of 30 to 100 m x 10^{-6}.

The analysis of fluid flow over leaf surfaces in Chapter 4.1 quantifies the relationship between leaf size and fluid velocity. The difference between water movement inside and outside an enclosure will depend mainly upon the external water movement. That is, the internal water movement will be closely defined but that outside will be very variable and require measurement. The most widely adopted methods for measuring current velocities are meters with rotating elements and surface/subsurface floats. These require a lot of time and cannot be used at velocities lower than about 5 cm $sec.^{-1}$ (Pikush, 1971). He further states that in many reservoirs more than half the water moves at

velocities lower than 5 cm sec.$^{-1}$ and proposes the use of a pneumatic method for measurement of water currents. Current velocities in streams are usually faster (0·3 to 1·0 m sec.$^{-1}$) and can easily be measured (Ladle and Casey, 1971). Attempts to measure under-water currents in Lochs Croispol and Borralie were made but were unsuccessful.

Langmuir circulations can be involved in mixing lakes (Scott, Stewart and Coinvestigators, 1968). They analysed the relation between wind speed and descending water currents. A similar analysis was performed by Faller (1971) on oceanic langmuir circulations and he noted that wind speeds of 4 m sec.$^{-1}$ produced strong descending currents. He concludes that in general molecular diffusion processes may be ignored in comparison with eddy diffusion. Murphy (1972) calculated that eddy diffusivities in the coastal currents of lakes are low. This was taken to mean that steady and uniform currents are not good dispersers of effluents. But he observed that steady and uniform currents rarely persist for more than a few hours and in the normal course of events the current changes vigorously or ceases entirely. Dyes released in very slow currents hugged the shoreline for several days, forming a stagnant pool with very little mixing.

Wave formation in lochs will contribute to flow around the loch. The depth of the wave mixed layer is

usually taken to be half the wavelength. Smith and Sinclair (1972) show that this is a reasonable approximation of Loch Leven, Fife. They observed that with larger fetches and wind speeds the depth of the wave mixed layer is appreciable and may mask the motion due to steady currents, even at depths of 10 metres. The distortion of the orbital motion of the water particles in waves is important in the circulation of lochs. The return motion of the particle beneath the wave is slightly less than the forward motion and net forward movement occurs. Jones (1970) found mean water velocities of 0·7 to 16 m per minute at a depth of 2 metres in Lake Huron.

Sokolov (1972) states that underground inflow in lakes is extremely difficult to compute, is inadequately studied, and is therefore often neglected. The existence of underwater springs in Lochs Croispol and Borralie was suspected and evidence for their existence was searched for while scuba diving but none could be found.

Thus, the mixing of water in lakes is complicated (Simons, 1971) and would be very difficult to reproduce in an enclosure. The experimenter can adopt two approaches to overcome this problem. Firstly, to use a constant velocity of fluid by pumping it around the chamber. Although this approach is very convenient in the laboratory it is very difficult to achieve <u>in situ</u> with the required replication. Secondly, no currents

can be provided and to rely upon convection currents for mixing. This technique is usually adopted for *in situ* enclosures. It will, therefore, be expected that enclosure under these conditions will tend to produce local stagnation of carbon next to the leaf surface. It would be possible to agitate the contents of the enclosure by magnetic stirring, mechanical stirring, or shaking the enclosure but these methods would also be difficult *in situ* .

The turbulent flow experiments (Chapter 4.4) were performed in enclosures that could be set up for use in an *in situ* experiment. The concentration of carbon in the incubating fluid was 2×10^{-2} moles per litre which is nearly an order of magnitude greater than that in calcareous waters. The results in Table 4.26 show that the difference between flow and no flow treatments on the uptake of carbon in the light was about 40%. The effect on the carbon uptake in the dark was even greater at about 65%. These effects were observed whether or not the leaves were attached or detached from the shoot before the start of the experiment. It can, therefore, be concluded that the effect of flow in enclosures is very significant at controlling the rate of photosynthesis when other factors should be non-limiting.

These enclosures were significantly larger than those often used for *in situ* experiments and the leaves were able to flap. This is a situation that probably

aided photosynthesis in addition to flow (Chapter 4.1).

The laminar flow experiments (Chapter 4.5) were conducted in a more precisely controlled flow environment than the turbulent flow experiments. In order to achieve reproducable conditions, it was necessary to have only one tissue sample in a chamber at a time and this made it more difficult to achieve enough replication.

The problem of variable leaf geometry was eliminated in a crude manner by using discs cut from leaves. These were as large as could be reasonably be obtained from the leaves of P. praelongus used and measured 2 cm in diameter. These discs were held by two hooked needles in such a manner as to produce minimum interference with the flow of incubating medium (Figure 4.23a). The discs were not observed to flap during any of the experiments.

Two series of experiments were performed, each at a different external carbon concentration. The results (Table 4.27 and 4.28) show that the higher flow rate produces a higher uptake at each carbon concentration. The statistical insignificance at the higher concentration is likely to be due to the lack of replication. However, the percentage increases in uptake at the higher flow velocities are 30% and 40% for the lower and higher carbon concentrations respectively. This compares with the difference found in the static/turbulent flow increase in uptake.

This is, therefore, convincing evidence that the effect of enclosure of aquatic leaves and the consequent reduction of water movement on them will have a significant influence on the subsequent rates of measured ^{14}C incorporation. Recently, Littler (1979) also implicated the involvement of water movement in enclosures on apparent photosynthesis rates in marine algae. Therefore, water movement must be defined inside enclosures used for productivity estimates.

5.5 Diffusion of Carbon Dioxide in the Aquatic Environment

As the diffusivity of carbon dioxide in water is four orders of magnitude less than it is in air, any factors affecting the diffusive supply of carbon dioxide will be important. Gaastra (1959) indicates that under light saturation and at normal carbon dioxide concentrations the rate of diffusion of carbon dioxide determines the rate of photosynthesis in crop plants. Szeicz and Yabuki (1964) showed that the dry matter production of crop plants in glasshouses can be greatly increased if additional carbon dioxide is supplied, provided the rate of photosynthesis is not limited by low levels of light intensity. Meidner (1969) explains this in terms of the movement of dissolved carbon dioxide in the liquid state and suggests that this is not diffusional but is aided by streaming and enzyme action. Stomatal resistances, however, largely predominate in controlling the rate of photosynthesis. Leaves of P. perfoliatus and P. Praelongus have non functional residual stomata and carbon dioxide will have to diffuse through the cuticle to reach the cell walls. The thickness of the cuticle of Potamogeton species is not known but is below the resolution of the light microscope (Schonherr 1976). He found that the cuticular membranes of the submerged leaves of Potamogeton are very different from those of terrestrial plants and they were shown to restrict the diffusion of water to a much lower

degree. This high permeability of the Potamogeton cuticle may be due to the absence of cuticular waxes.

Plants can adapt to different levels of carbon dioxide in the atmosphere. Alpine plants have been shown to have higher rates of photosynthesis than Arctic, sea level, members of the same species, at various carbon dioxide concentrations (Billings, Clebsch and Mooney, 1961). They suggest the ability to utilise carbon dioxide effectively at low concentrations may be involved in the survival of the plants at high altitude. Bristow (1968) showed that increasing the carbon dioxide concentration on the terrestrial form of Marsilea had the striking effect of causing the plants to develop many of the characteristics of the water form. This involved a reduction in the number of stomata on the lower side of the leaf and internodal elongation. The effect was the same whether in the light or the dark. Gaudet (1968) suggests that it appears that leaf form in aquatic plants responds to changes in metabolism which in turn occur in response to environmental cues.

Adaptions to an aquatic leaf that would be advantageous to the supply of carbon must be considered. Larger leaf sizes and lower flow rates will be disadvantageous. The broad leaved Potamogeton species such as perfoliatus and praelongus have leaves that are predominantly three cells thick. They might be considered to have adapted to a reduction in the internal diffusive

resistance (Chapter 4.1) compared to that which would be offered by a terrestrial leaf under water. However, the internal diffusive resistance may be expected to be small compared to the external resistance (Chapter 4.2) and the external concentration of carbon dioxide around the leaf may be depleted. This would be a different adaption to that of Myriophyllum which has finely divided leaves, an effect that would decrease the external diffusive resistance.

The results of the diffusion analysis across the empirical boundary layer in the laminar flow experiments in Chapter 4 section 5 showed that the diffusion of free carbon dioxide alone would not be sufficient to support the measured fluxes of ^{14}C. Therefore, bicarbonate must be considered as an additional source of inorganic carbon in these experiments. However, the thickness of the boundary layer used in the calculations was theoretical not empirical and this conclusion would be reinforced if the thickness of the boundary layer could be measured directly.

5.6 Summary

Light and dark ^{14}C incorporation rates of two broad-leaved pondweeds, Potamogeton perfoliatus and P. praelongus, were measured in the laboratory using procedures developed for use during in situ field productivity estimates. These measurements are used to evaluate the errors involved in the estimation of photosynthesis rates by this method and to provide recommendations for reducing these errors.

Large variations in the ^{14}C uptake, of replicate leaves or cut discs, were correlated with the position of the leaf on the stem and with the position of the disc on the leaf. Variation due to the size of the disc and to the effect of cutting were much less pronounced.

Measurement of the rate of release of previously incorporated ^{14}C from leaves showed that the release of carbon dioxide is greater in the dark than in the light, suggesting that extensive refixation is occurring and that this method will overestimate net photosynthesis and underestimate gross photosynthesis in light/dark bottle experiments.

The movement of $^{14}CO_2$ from the roots to the leaves of a plant was shown to be small and it is concluded that this may be disregarded as a significant source of carbon dioxide for photosynthesis.

The ratio of leaf area to fluid volume in experimental enclosures was shown to correlate with the size of the pH changes caused by photosynthesis. Changes in pH occurring during typical *in situ* experiments were shown to be significantly larger than those occurring naturally and it is recommended that large enclosures with small quantities of leaf tissue are used.

Reynolds number calculations show that laminar boundary layers might be expected to predominate for broad leaves in both the aquatic and terrestrial situation. Theoretical boundary layer thicknesses, for leaves of similar sizes at similar bulk fluid velocities, show that the laminar boundary layer in water will be approximately four times less than that in air.

Turbulent flow produced increases of more than 40% in measured ^{14}C incorporation over unstirred enclosures. Different laminar flow rates over the surface of leaf discs produced measurable changes in the rate of ^{14}C incorporation, showing a correlation between laminar boundary layer thickness and the rate of $^{14}CO_2$ uptake. These measurements show that the diffusion of free carbon dioxide across the average laminar boundary layer would not be fast enough to support the flux of ^{14}C, which must be assisted by the diffusion of the bicarbonate ion.

GLOSSARY

$(CO_2)_{BULK}$ = concentration of CO_2 in the bulk solution.

$(CO_2)_{CHL}$ = concentration of CO_2 at the chloroplast.

$(CO_2)_{LEAF}$ = concentration of CO_2 at the leaf surface.

cpm = counts per minute.

cpm-bg = counts per minute minus background counts.

D_{CO_2} = diffusity of CO_2 .

d = depth of laminar boundary layer.

$d_{1/2}$ = sample thickness that will stop half the incident beta particles.

d_{av} = average boundary layer thickness.

d_{SL} = depth of laminar sublayer.

d_T = depth of turbulent boundary layer.

E = total alkalinity in milliequivalents per litre.

E_{max} = maximum energy of beta particles.

F_{CO_2} = flux of CO_2 .

i = internal diffusion path.

in situ = in its (original) place.

K_1 = apparent dissociation constant for the first ionization step of carbonic acid.

K_2 = second dissociation constant of carbonic acid.

L = distance from the leading edge of a surface.

L_T = distance from onset of turbulence.

n_o = observed cpm.

n_t = true cpm.

R_e = Reynolds number.

R_{eL} = Reynolds number at distance L .

R_{eLT} = Reynolds number at distance LT .

S.L.A. = specific leaf area.

T.A. = total alkalinity.

T.C. = total carbonic acid.

T.D.S. = total dissolved solids.

U = bulk fluid velocity.

V = kinematic viscosity.

v = volume of boundary layer over a surface.

x = sample thickness in mg cm^{-2}.

Y = univalent activity coefficient

α = gas solubility coefficient.

μ = dynamic viscosity.

ρ = density.

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APPENDIX I

A set of tables, for different values of total alkalinity (E), showing the relationship between the free carbon dioxide concentration ($M \times 10^{-3}\ L^{-1}$), the pH, and the univalent activity coefficient Y, for natural waters according to the equation (Kemp 1971).

$$\text{Free carbon dioxide} = \frac{H^2.Y^6}{K_1.H.Y + 2.K_1.K_2}$$

```
 TABLE FOR  E=  6.36[illegible]E-01

 Y=    .940E 00 .938E 00 .936E 00 .934E 00 .932E 00 .930E 00 .922E 00 .904E 00 .894E 00 .885E 00

 8.00 .710E-01 .706E-01 .703E-01 .700E-01 .697E-01 .694E-01 .682E-01 .655E-01 .640E-01 .627E-01
 8.01 .693E-01 .690E-01 .687E-01 .684E-01 .681E-01 .678E-01 .666E-01 .640E-01 .625E-01 .612E-01
 8.02 .677E-01 .674E-01 .671E-01 .668E-01 .665E-01 .663E-01 .651E-01 .625E-01 .611E-01 .598E-01
 8.03 .662E-01 .659E-01 .656E-01 .653E-01 .650E-01 .647E-01 .636E-01 .611E-01 .597E-01 .584E-01
 8.04 .646E-01 .644E-01 .641E-01 .639E-01 .635E-01 .632E-01 .621E-01 .596E-01 .583E-01 .571E-01
 8.05 .631E-01 .629E-01 .626E-01 .623E-01 .620E-01 .618E-01 .607E-01 .583E-01 .569E-01 .558E-01
 8.06 .617E-01 .614E-01 .612E-01 .609E-01 .606E-01 .603E-01 .593E-01 .569E-01 .556E-01 .545E-01
 8.07 .603E-01 .600E-01 .597E-01 .595E-01 .592E-01 .590E-01 .579E-01 .556E-01 .543E-01 .532E-01
 8.08 .589E-01 .586E-01 .584E-01 .581E-01 .578E-01 .576E-01 .566E-01 .543E-01 .531E-01 .520E-01
 8.09 .575E-01 .573E-01 .571E-01 .568E-01 .565E-01 .563E-01 .553E-01 .531E-01 .519E-01 .508E-01
 8.10 .562E-01 .559E-01 .557E-01 .555E-01 .552E-01 .550E-01 .540E-01 .518E-01 .507E-01 .496E-01
 8.11 .549E-01 .546E-01 .544E-01 .542E-01 .539E-01 .537E-01 .527E-01 .506E-01 .495E-01 .485E-01
 8.12 .536E-01 .534E-01 .532E-01 .529E-01 .527E-01 .525E-01 .515E-01 .495E-01 .483E-01 .473E-01
 8.13 .524E-01 .522E-01 .519E-01 .517E-01 .515E-01 .512E-01 .503E-01 .483E-01 .472E-01 .462E-01
 8.14 .512E-01 .509E-01 .507E-01 .505E-01 .503E-01 .501E-01 .492E-01 .472E-01 .461E-01 .452E-01
 8.15 .500E-01 .498E-01 .496E-01 .493E-01 .491E-01 .489E-01 .480E-01 .461E-01 .450E-01 .441E-01
 8.16 .488E-01 .486E-01 .484E-01 .482E-01 .480E-01 .478E-01 .469E-01 .450E-01 .440E-01 .431E-01
 8.17 .477E-01 .475E-01 .473E-01 .471E-01 .469E-01 .467E-01 .458E-01 .440E-01 .430E-01 .421E-01
 8.18 .466E-01 .464E-01 .462E-01 .460E-01 .458E-01 .456E-01 .448E-01 .430E-01 .420E-01 .411E-01
 8.19 .455E-01 .453E-01 .451E-01 .449E-01 .447E-01 .445E-01 .437E-01 .420E-01 .410E-01 .401E-01
 8.20 .445E-01 .443E-01 .441E-01 .439E-01 .437E-01 .435E-01 .427E-01 .410E-01 .400E-01 .392E-01
 8.21 .434E-01 .432E-01 .430E-01 .429E-01 .427E-01 .425E-01 .417E-01 .400E-01 .391E-01 .383E-01
 8.22 .424E-01 .422E-01 .420E-01 .419E-01 .417E-01 .415E-01 .407E-01 .391E-01 .382E-01 .374E-01
 8.23 .414E-01 .413E-01 .411E-01 .409E-01 .407E-01 .405E-01 .398E-01 .382E-01 .373E-01 .365E-01
 8.24 .405E-01 .403E-01 .401E-01 .399E-01 .398E-01 .396E-01 .389E-01 .373E-01 .364E-01 .357E-01
 8.25 .395E-01 .394E-01 .392E-01 .390E-01 .388E-01 .387E-01 .380E-01 .364E-01 .356E-01 .348E-01
 8.26 .386E-01 .384E-01 .383E-01 .381E-01 .379E-01 .378E-01 .371E-01 .356E-01 .348E-01 .340E-01
 8.27 .377E-01 .376E-01 .374E-01 .372E-01 .371E-01 .369E-01 .362E-01 .348E-01 .340E-01 .332E-01
 8.28 .368E-01 .367E-01 .365E-01 .364E-01 .362E-01 .360E-01 .354E-01 .339E-01 .332E-01 .325E-01
 8.29 .360E-01 .358E-01 .357E-01 .355E-01 .353E-01 .352E-01 .346E-01 .332E-01 .324E-01 .317E-01
 8.30 .351E-01 .350E-01 .348E-01 .347E-01 .345E-01 .344E-01 .337E-01 .324E-01 .316E-01 .310E-01
 8.31 .343E-01 .342E-01 .340E-01 .339E-01 .337E-01 .336E-01 .330E-01 .316E-01 .309E-01 .302E-01
 8.32 .335E-01 .334E-01 .332E-01 .331E-01 .329E-01 .328E-01 .322E-01 .309E-01 .302E-01 .295E-01
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 8.37 .298E-01 .297E-01 .295E-01 .294E-01 .293E-01 .291E-01 .286E-01 .274E-01 .268E-01 .262E-01
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 8.47 .235E-01 .234E-01 .233E-01 .232E-01 .231E-01 .230E-01 .225E-01 .216E-01 .211E-01 .206E-01
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 8.57 .185E-01 .184E-01 .183E-01 .182E-01 .182E-01 .181E-01 .177E-01 .170E-01 .166E-01 .162E-01
 8.58 .181E-01 .180E-01 .179E-01 .178E-01 .177E-01 .176E-01 .173E-01 .166E-01 .162E-01 .158E-01
 8.59 .176E-01 .176E-01 .175E-01 .174E-01 .173E-01 .172E-01 .169E-01 .162E-01 .158E-01 .154E-01
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 8.75 .120E-01 .119E-01 .119E-01 .118E-01 .117E-01 .117E-01 .114E-01 .109E-01 .107E-01 .104E-01
 8.76 .117E-01 .116E-01 .116E-01 .115E-01 .114E-01 .114E-01 .112E-01 .107E-01 .104E-01 .102E-01
 8.77 .114E-01 .113E- 1 .113E-01 .112E-01 .112E-01 .111E-01 .109E-01 .104E-01 .102E-01 .992E-02
 8.78 .111E-01 .111E-01 .110E-01 .110E-01 .109E-01 .108E-01 .106E-01 .102E-01 .990E-02 .967E-02
 8.79 .108E-01 .108E-01 .107E-01 .107E-01 .106E-01 .106E-01 .104E-01 .991E-02 .966E-02 .943E-02
 8.80 .106E-01 .105E-01 .105E-01 .104E-01 .104E-01 .103E-01 .101E-01 .967E-02 .942E-02 .920E-02
 8.81 .103E-01 .103E-01 .102E-01 .102E-01 .101E-01 .101E-01 .987E-02 .943E-02 .919E-02 .897E-02
 8.82 .101E-01 .100E-01 .997E-02 .992E-02 .987E-02 .982E-02 .963E-02 .920E-02 .896E-02 .875E-02
 8.83 .992E-02 .977E-02 .973E-02 .968E-02 .963E-02 .958E-02 .939E-02 .897E-02 .874E-02 .853E-02
 8.84 .958E-02 .954E-02 .949E-02 .944E-02 .940E-02 .935E-02 .916E-02 .875E-02 .852E-02 .832E-02
 8.85 .935E-02 .930E-02 .926E-02 .921E-02 .916E-02 .912E-02 .894E-02 .853E-02 .831E-02 .811E-02
 8.86 .912E-02 .907E-02 .903E-02 .898E-02 .894E-02 .889E-02 .872E-02 .832E-02 .810E-02 .791E-02
 8.87 .889E-02 .885E-02 .881E-02 .876E-02 .872E-02 .867E-02 .850E-02 .811E-02 .790E-02 .771E-02
 8.88 .868E-02 .864E- 2 .859E- 2 .855E-02 .850E-02 .846E-02 .829E-02 .791E-02 .770E-02 .752E-02
 8.89 .846E-02 .842E-02 .838E-02 .834E-02 .829E-02 .825E-02 .808E-02 .771E-02 .751E-02 .733E-02
 8.90 .825E-02 .821E-02 .817E-02 .813E-02 .809E-02 .805E-02 .788E-02 .752E-02 .732E-02 .715E-02
 8.91 .805E-02 .801E-02 .797E-02 .793E-02 .789E-02 .785E-02 .769E-02 .733E-02 .714E-02 .696E-02
 8.92 .785E-02 .781E-02 .777E-02 .773E-02 .769E-02 .765E-02 .750E-02 .715E-02 .696E-02 .679E-02
 8.93 .765E-02 .762E-02 .758E-02 .754E-02 .750E-02 .746E-02 .731E-02 .697E-02 .678E-02 .662E-02
 8.94 .746E-02 .743E-02 .739E-02 .735E-02 .731E-02 .728E-02 .713E-02 .679E-02 .661E-02 .645E-02
 8.95 .728E-02 .724E-02 .720E-02 .717E-02 .713E-02 .709E-02 .695E-02 .662E-02 .644E-02 .629E-02
 8.96 .710E-02 .706E-02 .703E-02 .699E-02 .695E-02 .692E-02 .677E-02 .646E-02 .628E-02 .612E-02
 8.97 .692E-02 .688E-02 .685E-02 .681E-02 .678E-02 .674E-02 .660E-02 .629E-02 .612E-02 .597E-02
 8.98 .675E-02 .671E-02 .668E-02 .664E-02 .661E-02 .657E-02 .644E-02 .613E-02 .596E-02 .582E-02
 8.99 .658E-02 .654E-02 .651E-02 .647E-02 .644E-02 .641E-02 .627E-02 .598E-02 .581E-02 .567E-02
 9.00 .641E-02 .638E-02 .634E-02 .631E-02 .628E-02 .625E-02 .611E-02 .582E-02 .566E-02 .552E-02
  STOP     0
/E                                                                                    21.15.34
```

TABLE FOR E= 6.35200E-01

Y=	.940E 00	.930E 00	.936E 00	.934E 00	.932E 00	.930E 00	.922E 00	.904E 00	.894E 00	.885E 00
8.00	.700E-01	.697E-01	.694E-01	.691E-01	.688E-01	.685E-01	.672E-01	.646E-01	.631E-01	.618E-01
8.01	.684E-01	.681E-01	.678E-01	.675E-01	.672E-01	.669E-01	.657E-01	.631E-01	.617E-01	.604E-01
8.02	.668E-01	.665E-01	.662E-01	.659E-01	.656E-01	.653E-01	.642E-01	.616E-01	.602E-01	.590E-01
8.03	.652E-01	.650E-01	.647E-01	.644E-01	.641E-01	.638E-01	.627E-01	.602E-01	.588E-01	.576E-01
8.04	.637E-01	.635E-01	.632E-01	.629E-01	.626E-01	.624E-01	.613E-01	.588E-01	.575E-01	.563E-01
8.05	.623E-01	.621E-01	.617E-01	.615E-01	.612E-01	.609E-01	.598E-01	.575E-01	.562E-01	.550E-01
8.06	.608E-01	.606E-01	.603E-01	.600E-01	.598E-01	.595E-01	.585E-01	.561E-01	.549E-01	.537E-01
8.07	.594E-01	.592E-01	.589E-01	.587E-01	.584E-01	.581E-01	.571E-01	.548E-01	.536E-01	.525E-01
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8.10	.554E-01	.552E-01	.549E-01	.547E-01	.544E-01	.542E-01	.532E-01	.511E-01	.500E-01	.489E-01
8.11	.541E-01	.539E-01	.537E-01	.534E-01	.532E-01	.529E-01	.520E-01	.499E-01	.488E-01	.478E-01
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8.32	.331E-01	.329E-01	.328E-01	.326E-01	.325E-01	.323E-01	.317E-01	.305E-01	.297E-01	.291E-01
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8.41	.267E-01	.266E-01	.265E-01	.264E-01	.262E-01	.261E-01	.256E-01	.246E-01	.240E-01	.235E-01
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8.51	.211E-01	.210E-01	.209E-01	.208E-01	.207E-01	.206E-01	.202E-01	.194E-01	.189E-01	.185E-01
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8.57	.182E-01	.182E-01	.181E-01	.180E-01	.179E-01	.178E-01	.175E-01	.168E-01	.163E-01	.160E-01
8.58	.178E-01	.177E-01	.176E-01	.176E-01	.175E-01	.174E-01	.171E-01	.164E-01	.160E-01	.156E-01
8.59	.174E-01	.173E-01	.172E-01	.171E-01	.171E-01	.170E-01	.167E-01	.160E-01	.156E-01	.152E-01
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8.66	.147E-01	.146E-01	.145E-01	.145E-01	.144E-01	.143E-01	.141E-01	.135E-01	.131E-01	.128E-01
8.67	.143E-01	.143E-01	.142E-01	.141E-01	.141E-01	.140E-01	.137E-01	.131E-01	.128E-01	.125E-01
8.68	.140E-01	.139E-01	.139E-01	.138E-01	.137E-01	.137E-01	.134E-01	.128E-01	.125E-01	.122E-01
8.69	.137E-01	.136E-01	.135E-01	.135E-01	.134E-01	.133E-01	.131E-01	.125E-01	.122E-01	.119E-01
8.70	.133E-01	.133E-01	.132E-01	.131E-01	.131E-01	.130E-01	.128E-01	.122E-01	.119E-01	.116E-01
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8.79	.107E-01	.106E-01	.106E-01	.105E-01	.105E-01	.104E-01	.102E-01	.977E-02	.952E-02	.930E-02
8.80	.104E-01	.104E-01	.103E-01	.103E-01	.102E-01	.102E-01	.998E-02	.953E-02	.929E-02	.907E-02
8.81	.102E-01	.101E-01	.101E-01	.100E-01	.998E-02	.993E-02	.974E-02	.930E-02	.906E-02	.885E-02
8.82	.993E-02	.988E-02	.983E-02	.978E-02	.974E-02	.969E-02	.950E-02	.907E-02	.884E-02	.863E-02
8.83	.969E-02	.964E-02	.959E-02	.954E-02	.950E-02	.945E-02	.926E-02	.885E-02	.862E-02	.841E-02
8.84	.945E-02	.940E-02	.936E-02	.931E-02	.926E-02	.922E-02	.904E-02	.863E-02	.840E-02	.820E-02
8.85	.922E-02	.917E-02	.913E-02	.908E-02	.904E-02	.899E-02	.881E-02	.841E-02	.820E-02	.800E-02
8.86	.899E-02	.895E-02	.890E-02	.886E-02	.881E-02	.877E-02	.860E-02	.821E-02	.799E-02	.780E-02
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8.89	.834E-02	.830E-02	.826E-02	.822E-02	.818E-02	.814E-02	.797E-02	.761E-02	.741E-02	.723E-02
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8.91	.794E-02	.791E-02	.786E-02	.782E-02	.778E-02	.774E-02	.758E-02	.723E-02	.704E-02	.687E-02
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8.93	.755E-02	.751E-02	.747E-02	.743E-02	.740E-02	.736E-02	.721E-02	.687E-02	.669E-02	.653E-02
8.94	.736E-02	.732E-02	.729E-02	.725E-02	.721E-02	.717E-02	.703E-02	.670E-02	.652E-02	.636E-02
8.95	.718E-02	.714E-02	.711E-02	.707E-02	.703E-02	.699E-02	.685E-02	.653E-02	.635E-02	.620E-02
8.96	.700E-02	.696E-02	.693E-02	.689E-02	.686E-02	.682E-02	.668E-02	.637E-02	.619E-02	.604E-02
8.97	.682E-02	.679E-02	.675E-02	.672E-02	.668E-02	.665E-02	.651E-02	.620E-02	.604E-02	.589E-02
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8.03	.643E-01	.640E-01	.638E-01	.635E-01	.632E-01	.629E-01	.618E-01	.594E-01	.580E-01	.568E-01
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8.24	.394E-01	.392E-01	.390E-01	.388E-01	.387E-01	.385E-01	.378E-01	.363E-01	.354E-01	.347E-01
8.25	.384E-01	.383E-01	.381E-01	.379E-01	.378E-01	.376E-01	.369E-01	.354E-01	.346E-01	.339E-01
8.26	.375E-01	.374E-01	.372E-01	.370E-01	.369E-01	.367E-01	.361E-01	.346E-01	.338E-01	.331E-01
8.27	.367E-01	.365E-01	.363E-01	.362E-01	.360E-01	.359E-01	.352E-01	.338E-01	.330E-01	.323E-01
8.28	.358E-01	.357E-01	.355E-01	.353E-01	.352E-01	.350E-01	.344E-01	.330E-01	.322E-01	.316E-01
8.29	.350E-01	.348E-01	.347E-01	.345E-01	.344E-01	.342E-01	.336E-01	.322E-01	.315E-01	.308E-01
8.30	.342E-01	.340E-01	.339E-01	.337E-01	.336E-01	.334E-01	.328E-01	.315E-01	.307E-01	.301E-01
8.31	.334E-01	.332E-01	.331E-01	.329E-01	.328E-01	.326E-01	.320E-01	.307E-01	.300E-01	.294E-01
8.32	.326E-01	.325E-01	.323E-01	.322E-01	.320E-01	.319E-01	.313E-01	.300E-01	.293E-01	.287E-01
8.33	.318E-01	.317E-01	.316E-01	.314E-01	.313E-01	.311E-01	.306E-01	.293E-01	.286E-01	.280E-01
8.34	.311E-01	.310E-01	.308E-01	.307E-01	.305E-01	.304E-01	.299E-01	.286E-01	.280E-01	.274E-01
8.35	.304E-01	.302E-01	.301E-01	.300E-01	.298E-01	.297E-01	.292E-01	.280E-01	.273E-01	.267E-01
8.36	.297E-01	.295E-01	.294E-01	.293E-01	.291E-01	.290E-01	.285E-01	.273E-01	.267E-01	.261E-01
8.37	.290E-01	.289E-01	.287E-01	.286E-01	.284E-01	.283E-01	.278E-01	.267E-01	.260E-01	.255E-01
8.38	.283E-01	.282E-01	.280E-01	.279E-01	.278E-01	.277E-01	.272E-01	.260E-01	.254E-01	.249E-01
8.39	.276E-01	.275E-01	.274E-01	.273E-01	.271E-01	.270E-01	.265E-01	.254E-01	.248E-01	.243E-01
8.40	.270E-01	.269E-01	.267E-01	.266E-01	.265E-01	.264E-01	.259E-01	.248E-01	.242E-01	.237E-01
8.41	.263E-01	.262E-01	.261E-01	.260E-01	.259E-01	.258E-01	.253E-01	.242E-01	.237E-01	.232E-01
8.42	.257E-01	.256E-01	.255E-01	.254E-01	.253E-01	.252E-01	.247E-01	.237E-01	.231E-01	.226E-01
8.43	.251E-01	.250E-01	.249E-01	.248E-01	.247E-01	.246E-01	.241E-01	.231E-01	.226E-01	.221E-01
8.44	.245E-01	.244E-01	.243E-01	.242E-01	.241E-01	.240E-01	.235E-01	.226E-01	.220E-01	.216E-01
8.45	.240E-01	.239E-01	.237E-01	.236E-01	.235E-01	.234E-01	.230E-01	.220E-01	.215E-01	.210E-01
8.46	.234E-01	.233E-01	.232E-01	.231E-01	.230E-01	.229E-01	.224E-01	.215E-01	.210E-01	.206E-01
8.47	.228E-01	.227E-01	.226E-01	.225E-01	.224E-01	.223E-01	.219E-01	.210E-01	.205E-01	.201E-01
8.48	.223E-01	.222E-01	.221E-01	.220E-01	.219E-01	.218E-01	.214E-01	.205E-01	.200E-01	.196E-01
8.49	.218E-01	.217E-01	.216E-01	.215E-01	.214E-01	.213E-01	.209E-01	.200E-01	.195E-01	.191E-01
8.50	.213E-01	.212E-01	.211E-01	.210E-01	.209E-01	.208E-01	.204E-01	.195E-01	.191E-01	.187E-01
8.51	.208E-01	.207E-01	.206E-01	.205E-01	.204E-01	.203E-01	.199E-01	.191E-01	.186E-01	.182E-01
8.52	.203E-01	.202E-01	.201E-01	.200E-01	.199E-01	.198E-01	.194E-01	.186E-01	.182E-01	.178E-01
8.53	.198E-01	.197E-01	.196E-01	.195E-01	.194E-01	.193E-01	.190E-01	.182E-01	.178E-01	.174E-01
8.54	.193E-01	.192E-01	.192E-01	.191E-01	.190E-01	.189E-01	.185E-01	.178E-01	.173E-01	.169E-01
8.55	.189E-01	.188E-01	.187E-01	.186E-01	.185E-01	.184E-01	.181E-01	.173E-01	.169E-01	.165E-01
8.56	.184E-01	.183E-01	.183E-01	.182E-01	.181E-01	.180E-01	.177E-01	.169E-01	.165E-01	.161E-01
8.57	.180E-01	.179E-01	.178E-01	.177E-01	.177E-01	.176E-01	.172E-01	.165E-01	.161E-01	.158E-01
8.58	.176E-01	.175E-01	.174E-01	.173E-01	.172E-01	.172E-01	.168E-01	.161E-01	.157E-01	.154E-01
8.59	.171E-01	.171E-01	.170E-01	.169E-01	.168E-01	.167E-01	.164E-01	.157E-01	.154E-01	.150E-01
8.60	.167E-01	.167E-01	.166E-01	.165E-01	.164E-01	.163E-01	.160E-01	.154E-01	.150E-01	.147E-01
8.61	.163E-01	.163E-01	.162E-01	.161E-01	.160E-01	.160E-01	.157E-01	.150E-01	.146E-01	.143E-01
8.62	.159E-01	.159E-01	.158E-01	.157E-01	.157E-01	.156E-01	.153E-01	.146E-01	.143E-01	.140E-01
8.63	.156E-01	.155E-01	.154E-01	.154E-01	.153E-01	.152E-01	.149E-01	.143E-01	.139E-01	.136E-01
8.64	.152E-01	.151E-01	.151E-01	.150E-01	.149E-01	.148E-01	.146E-01	.139E-01	.136E-01	.133E-01
8.65	.148E-01	.148E-01	.147E-01	.146E-01	.146E-01	.145E-01	.142E-01	.136E-01	.133E-01	.130E-01
8.66	.145E-01	.144E-01	.143E-01	.143E-01	.142E-01	.141E-01	.139E-01	.133E-01	.129E-01	.127E-01
8.67	.141E-01	.141E-01	.140E-01	.139E-01	.139E-01	.138E-01	.135E-01	.130E-01	.126E-01	.123E-01
8.68	.138E-01	.137E-01	.137E-01	.136E-01	.135E-01	.135E-01	.132E-01	.126E-01	.123E-01	.120E-01
8.69	.135E-01	.134E-01	.133E-01	.133E-01	.132E-01	.131E-01	.129E-01	.123E-01	.120E-01	.118E-01
8.70	.131E-01	.131E-01	.130E-01	.130E-01	.129E-01	.128E-01	.126E-01	.120E-01	.117E-01	.115E-01
8.71	.128E-01	.128E-01	.127E-01	.126E-01	.126E-01	.125E-01	.123E-01	.117E-01	.115E-01	.112E-01
8.72	.125E-01	.125E-01	.124E-01	.123E-01	.123E-01	.122E-01	.120E-01	.115E-01	.112E-01	.109E-01
8.73	.122E-01	.122E-01	.121E-01	.120E-01	.120E-01	.119E-01	.117E-01	.112E-01	.109E-01	.106E-01
8.74	.119E-01	.119E-01	.118E-01	.117E-01	.117E-01	.116E-01	.114E-01	.109E-01	.106E-01	.104E-01
8.75	.116E-01	.116E-01	.115E-01	.115E-01	.114E-01	.114E-01	.111E-01	.106E-01	.104E-01	.101E-01
8.76	.113E-01	.113E-01	.112E-01	.112E-01	.111E-01	.111E-01	.109E-01	.104E-01	.101E-01	.988E-02
8.77	.111E-01	.110E-01	.110E-01	.109E-01	.109E-01	.108E-01	.106E-01	.101E-01	.987E-02	.964E-02
8.78	.108E-01	.108E-01	.107E-01	.106E-01	.106E-01	.105E-01	.103E-01	.988E-02	.963E-02	.940E-02
8.79	.105E-01	.105E-01	.104E-01	.104E-01	.103E-01	.103E-01	.101E-01	.964E-02	.939E-02	.917E-02
8.80	.103E-01	.102E-01	.102E-01	.101E-01	.101E-01	.100E-01	.984E-02	.940E-02	.916E-02	.894E-02
8.81	.100E-01	.999E-02	.994E-02	.989E-02	.984E-02	.979E-02	.960E-02	.917E-02	.893E-02	.872E-02
8.82	.979E-02	.974E-02	.969E-02	.965E-02	.960E-02	.955E-02	.936E-02	.894E-02	.871E-02	.851E-02
8.83	.955E-02	.950E-02	.946E-02	.941E-02	.936E-02	.932E-02	.913E-02	.872E-02	.850E-02	.830E-02
8.84	.932E-02	.927E-02	.923E-02	.919E-02	.913E-02	.909E-02	.891E-02	.851E-02	.829E-02	.809E-02
8.85	.909E-02	.904E-02	.900E-02	.896E-02	.891E-02	.887E-02	.869E-02	.830E-02	.808E-02	.789E-02
8.86	.887E-02	.882E-02	.878E-02	.873E-02	.869E-02	.865E-02	.847E-02	.809E-02	.788E-02	.769E-02
8.87	.865E-02	.860E-02	.856E-02	.852E-02	.848E-02	.843E-02	.826E-02	.789E-02	.768E-02	.750E-02
8.88	.843E-02	.839E-02	.835E-02	.831E-02	.827E-02	.823E-02	.806E-02	.769E-02	.749E-02	.731E-02
8.89	.823E-02	.819E-02	.814E-02	.810E-02	.806E-02	.802E-02	.786E-02	.750E-02	.730E-02	.713E-02
8.90	.802E-02	.798E-02	.794E-02	.790E-02	.786E-02	.782E-02	.767E-02	.731E-02	.712E-02	.695E-02
8.91	.782E-02	.779E-02	.775E-02	.771E-02	.767E-02	.763E-02	.747E-02	.713E-02	.694E-02	.677E-02
8.92	.763E-02	.759E-02	.755E-02	.752E-02	.748E-02	.744E-02	.729E-02	.695E-02	.677E-02	.660E-02
8.93	.744E-02	.740E-02	.737E-02	.733E-02	.729E-02	.725E-02	.711E-02	.678E-02	.659E-02	.643E-02
8.94	.726E-02	.722E-02	.718E-02	.715E-02	.711E-02	.707E-02	.693E-02	.661E-02	.643E-02	.627E-02
8.95	.708E-02	.704E-02	.701E-02	.697E-02	.693E-02	.690E-02	.675E-02	.644E-02	.627E-02	.611E-02
8.96	.690E-02	.686E-02	.683E-02	.679E-02	.676E-02	.672E-02	.658E-02	.628E-02	.611E-02	.595E-02
8.97	.673E-02	.669E-02	.666E-02	.662E-02	.659E-02	.656E-02	.642E-02	.612E-02	.595E-02	.580E-02
8.98	.656E-02	.652E-02	.649E-02	.646E-02	.642E-02	.639E-02	.626E-02	.596E-02	.580E-02	.565E-02
8.99	.639E-02	.636E-02	.633E-02	.629E-02	.626E-02	.623E-02	.610E-02	.581E-02	.565E-02	.551E-02
9.00	.623E-02	.620E-02	.617E-02	.614E-02	.610E-02	.607E-02	.594E-02	.566E-02	.551E-02	.537E-02

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Y=	.940E 00	.938E 00	.936E 00	.934E 00	.932E 00	.930E 00	.922E 00	.904E 00	.894E 00	.885E 00
8.00	.688E-01	.677E-01	.674E-01	.671E-01	.668E-01	.665E-01	.654E-01	.628E-01	.613E-01	.601E-01
8.01	.664E-01	.661E-01	.659E-01	.656E-01	.653E-01	.650E-01	.638E-01	.613E-01	.599E-01	.587E-01
8.02	.649E-01	.646E-01	.643E-01	.641E-01	.638E-01	.635E-01	.624E-01	.599E-01	.585E-01	.573E-01
8.03	.634E-01	.631E-01	.629E-01	.626E-01	.623E-01	.620E-01	.609E-01	.585E-01	.572E-01	.560E-01
8.04	.619E-01	.617E-01	.614E-01	.611E-01	.609E-01	.606E-01	.595E-01	.572E-01	.559E-01	.547E-01
8.05	.605E-01	.603E-01	.600E-01	.597E-01	.595E-01	.592E-01	.582E-01	.558E-01	.546E-01	.534E-01
8.06	.591E-01	.589E-01	.586E-01	.583E-01	.581E-01	.578E-01	.568E-01	.546E-01	.533E-01	.522E-01
8.07	.578E-01	.575E-01	.573E-01	.570E-01	.567E-01	.565E-01	.555E-01	.533E-01	.521E-01	.510E-01
8.08	.564E-01	.562E-01	.559E-01	.557E-01	.554E-01	.552E-01	.542E-01	.521E-01	.509E-01	.498E-01
8.09	.551E-01	.549E-01	.546E-01	.544E-01	.542E-01	.539E-01	.530E-01	.509E-01	.497E-01	.487E-01
8.10	.538E-01	.536E-01	.534E-01	.531E-01	.529E-01	.527E-01	.517E-01	.497E-01	.485E-01	.475E-01
8.11	.526E-01	.524E-01	.521E-01	.519E-01	.517E-01	.515E-01	.505E-01	.485E-01	.474E-01	.464E-01
8.12	.514E-01	.512E-01	.509E-01	.507E-01	.505E-01	.503E-01	.494E-01	.474E-01	.463E-01	.454E-01
8.13	.502E-01	.500E-01	.498E-01	.495E-01	.493E-01	.491E-01	.482E-01	.463E-01	.452E-01	.443E-01
8.14	.490E-01	.488E-01	.486E-01	.484E-01	.482E-01	.480E-01	.471E-01	.452E-01	.442E-01	.433E-01
8.15	.479E-01	.477E-01	.475E-01	.473E-01	.471E-01	.469E-01	.460E-01	.442E-01	.432E-01	.423E-01
8.16	.468E-01	.466E-01	.464E-01	.462E-01	.460E-01	.458E-01	.450E-01	.432E-01	.422E-01	.413E-01
8.17	.457E-01	.455E-01	.453E-01	.451E-01	.449E-01	.447E-01	.439E-01	.422E-01	.412E-01	.403E-01
8.18	.447E-01	.445E-01	.443E-01	.441E-01	.439E-01	.437E-01	.429E-01	.412E-01	.402E-01	.394E-01
8.19	.436E-01	.434E-01	.432E-01	.430E-01	.429E-01	.427E-01	.419E-01	.402E-01	.393E-01	.385E-01
8.20	.426E-01	.424E-01	.422E-01	.420E-01	.419E-01	.417E-01	.409E-01	.393E-01	.384E-01	.376E-01
8.21	.416E-01	.414E-01	.413E-01	.411E-01	.409E-01	.407E-01	.400E-01	.384E-01	.375E-01	.367E-01
8.22	.407E-01	.405E-01	.403E-01	.401E-01	.399E-01	.398E-01	.391E-01	.375E-01	.366E-01	.358E-01
8.23	.397E-01	.395E-01	.394E-01	.392E-01	.390E-01	.388E-01	.381E-01	.366E-01	.358E-01	.350E-01
8.24	.388E-01	.386E-01	.384E-01	.383E-01	.381E-01	.379E-01	.373E-01	.358E-01	.349E-01	.342E-01
8.25	.379E-01	.377E-01	.376E-01	.374E-01	.372E-01	.371E-01	.364E-01	.349E-01	.341E-01	.334E-01
8.26	.370E-01	.368E-01	.367E-01	.365E-01	.364E-01	.362E-01	.355E-01	.341E-01	.333E-01	.326E-01
8.27	.361E-01	.360E-01	.358E-01	.357E-01	.355E-01	.353E-01	.347E-01	.333E-01	.325E-01	.319E-01
8.28	.353E-01	.351E-01	.350E-01	.348E-01	.347E-01	.345E-01	.339E-01	.325E-01	.318E-01	.311E-01
8.29	.345E-01	.343E-01	.342E-01	.340E-01	.339E-01	.337E-01	.331E-01	.318E-01	.310E-01	.304E-01
8.30	.337E-01	.335E-01	.334E-01	.332E-01	.331E-01	.329E-01	.323E-01	.310E-01	.303E-01	.297E-01
8.31	.329E-01	.327E-01	.326E-01	.325E-01	.323E-01	.322E-01	.316E-01	.303E-01	.296E-01	.290E-01
8.32	.321E-01	.320E-01	.318E-01	.317E-01	.316E-01	.314E-01	.309E-01	.296E-01	.289E-01	.283E-01
8.33	.314E-01	.312E-01	.311E-01	.310E-01	.308E-01	.307E-01	.301E-01	.289E-01	.282E-01	.276E-01
8.34	.306E-01	.305E-01	.304E-01	.302E-01	.301E-01	.300E-01	.294E-01	.282E-01	.276E-01	.270E-01
8.35	.299E-01	.298E-01	.297E-01	.295E-01	.294E-01	.293E-01	.287E-01	.276E-01	.269E-01	.263E-01
8.36	.292E-01	.291E-01	.290E-01	.288E-01	.287E-01	.286E-01	.281E-01	.269E-01	.263E-01	.257E-01
8.37	.286E-01	.284E-01	.283E-01	.282E-01	.280E-01	.279E-01	.274E-01	.263E-01	.257E-01	.251E-01
8.38	.279E-01	.278E-01	.276E-01	.275E-01	.274E-01	.273E-01	.268E-01	.257E-01	.251E-01	.245E-01
8.39	.272E-01	.271E-01	.270E-01	.269E-01	.267E-01	.266E-01	.261E-01	.251E-01	.245E-01	.239E-01
8.40	.266E-01	.265E-01	.264E-01	.262E-01	.261E-01	.260E-01	.255E-01	.245E-01	.239E-01	.234E-01
8.41	.260E-01	.259E-01	.257E-01	.256E-01	.255E-01	.254E-01	.249E-01	.239E-01	.233E-01	.228E-01
8.42	.254E-01	.252E-01	.251E-01	.250E-01	.249E-01	.248E-01	.243E-01	.233E-01	.228E-01	.223E-01
8.43	.248E-01	.247E-01	.245E-01	.244E-01	.243E-01	.242E-01	.238E-01	.228E-01	.222E-01	.218E-01
8.44	.242E-01	.241E-01	.240E-01	.239E-01	.237E-01	.236E-01	.232E-01	.222E-01	.217E-01	.213E-01
8.45	.236E-01	.235E-01	.234E-01	.233E-01	.232E-01	.231E-01	.227E-01	.217E-01	.212E-01	.207E-01
8.46	.231E-01	.230E-01	.229E-01	.227E-01	.226E-01	.225E-01	.221E-01	.212E-01	.207E-01	.203E-01
8.47	.225E-01	.224E-01	.223E-01	.222E-01	.221E-01	.220E-01	.216E-01	.207E-01	.202E-01	.198E-01
8.48	.220E-01	.219E-01	.218E-01	.217E-01	.216E-01	.215E-01	.211E-01	.202E-01	.197E-01	.193E-01
8.49	.215E-01	.214E-01	.213E-01	.212E-01	.211E-01	.210E-01	.206E-01	.197E-01	.193E-01	.188E-01
8.50	.210E-01	.209E-01	.208E-01	.207E-01	.206E-01	.205E-01	.201E-01	.193E-01	.188E-01	.184E-01
8.51	.205E-01	.204E-01	.203E-01	.202E-01	.201E-01	.200E-01	.196E-01	.188E-01	.184E-01	.180E-01
8.52	.200E-01	.199E-01	.198E-01	.197E-01	.196E-01	.195E-01	.192E-01	.184E-01	.179E-01	.175E-01
8.53	.195E-01	.194E-01	.193E-01	.192E-01	.192E-01	.191E-01	.187E-01	.179E-01	.175E-01	.171E-01
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8.55	.186E-01	.185E-01	.184E-01	.183E-01	.183E-01	.182E-01	.178E-01	.171E-01	.167E-01	.163E-01
8.56	.182E-01	.181E-01	.180E-01	.179E-01	.178E-01	.177E-01	.174E-01	.167E-01	.163E-01	.159E-01
8.57	.177E-01	.176E-01	.176E-01	.175E-01	.174E-01	.173E-01	.170E-01	.163E-01	.159E-01	.155E-01
8.58	.173E-01	.172E-01	.171E-01	.171E-01	.170E-01	.169E-01	.166E-01	.159E-01	.155E-01	.152E-01
8.59	.169E-01	.168E-01	.167E-01	.167E-01	.166E-01	.165E-01	.162E-01	.155E-01	.151E-01	.148E-01
8.60	.165E-01	.164E-01	.163E-01	.163E-01	.162E-01	.161E-01	.158E-01	.151E-01	.148E-01	.144E-01
8.61	.161E-01	.160E-01	.160E-01	.159E-01	.158E-01	.157E-01	.154E-01	.148E-01	.144E-01	.141E-01
8.62	.157E-01	.156E-01	.156E-01	.155E-01	.154E-01	.154E-01	.151E-01	.144E-01	.141E-01	.138E-01
8.63	.153E-01	.153E-01	.152E-01	.151E-01	.151E-01	.150E-01	.147E-01	.141E-01	.137E-01	.134E-01
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8.65	.146E-01	.146E-01	.145E-01	.144E-01	.143E-01	.143E-01	.140E-01	.134E-01	.131E-01	.128E-01
8.66	.143E-01	.142E-01	.141E-01	.141E-01	.140E-01	.139E-01	.137E-01	.131E-01	.128E-01	.125E-01
8.67	.139E-01	.139E-01	.138E-01	.137E-01	.137E-01	.136E-01	.133E-01	.128E-01	.125E-01	.122E-01
8.68	.136E-01	.135E-01	.135E-01	.134E-01	.133E-01	.133E-01	.130E-01	.125E-01	.122E-01	.119E-01
8.69	.133E-01	.132E-01	.131E-01	.131E-01	.130E-01	.130E-01	.127E-01	.122E-01	.119E-01	.116E-01
8.70	.130E-01	.129E-01	.128E-01	.128E-01	.127E-01	.126E-01	.124E-01	.119E-01	.116E-01	.113E-01
8.71	.126E-01	.126E-01	.125E-01	.125E-01	.124E-01	.123E-01	.121E-01	.116E-01	.113E-01	.110E-01
8.72	.123E-01	.123E-01	.122E-01	.122E-01	.121E-01	.120E-01	.118E-01	.113E-01	.110E-01	.108E-01
8.73	.120E-01	.120E-01	.119E-01	.119E-01	.118E-01	.118E-01	.115E-01	.110E-01	.107E-01	.105E-01
8.74	.117E-01	.117E-01	.116E-01	.116E-01	.115E-01	.115E-01	.112E-01	.108E-01	.105E-01	.102E-01
8.75	.115E-01	.114E-01	.114E-01	.113E-01	.112E-01	.112E-01	.110E-01	.105E-01	.102E-01	.999E-02
8.76	.112E-01	.111E-01	.111E-01	.110E-01	.110E-01	.109E-01	.107E-01	.102E-01	.997E-02	.974E-02
8.77	.109E-01	.109E-01	.108E-01	.108E-01	.107E-01	.107E-01	.104E-01	.998E-02	.973E-02	.950E-02
8.78	.106E-01	.106E-01	.105E-01	.105E-01	.104E-01	.104E-01	.102E-01	.974E-02	.949E-02	.927E-02
8.79	.104E-01	.103E-01	.103E-01	.102E-01	.102E-01	.101E-01	.994E-02	.950E-02	.926E-02	.904E-02
8.80	.101E-01	.101E-01	.100E-01	.999E-02	.994E-02	.989E-02	.970E-02	.927E-02	.903E-02	.882E-02
8.81	.989E-02	.984E-02	.979E-02	.975E-02	.970E-02	.965E-02	.946E-02	.904E-02	.881E-02	.860E-02
8.82	.965E-02	.960E-02	.956E-02	.951E-02	.946E-02	.941E-02	.923E-02	.881E-02	.859E-02	.839E-02
8.83	.941E-02	.937E-02	.932E-02	.928E-02	.923E-02	.918E-02	.900E-02	.860E-02	.838E-02	.818E-02
8.84	.918E-02	.914E-02	.909E-02	.905E-02	.900E-02	.896E-02	.878E-02	.838E-02	.817E-02	.797E-02
8.85	.896E-02	.891E-02	.887E-02	.883E-02	.878E-02	.874E-02	.856E-02	.818E-02	.796E-02	.777E-02
8.86	.874E-02	.870E-02	.865E-02	.861E-02	.857E-02	.852E-02	.835E-02	.797E-02	.777E-02	.758E-02
8.87	.852E-02	.848E-02	.844E-02	.840E-02	.836E-02	.831E-02	.815E-02	.778E-02	.757E-02	.739E-02
8.88	.831E-02	.827E-02	.823E-02	.819E-02	.815E-02	.811E-02	.795E-02	.758E-02	.738E-02	.721E-02
8.89	.811E-02	.807E-02	.803E-02	.799E-02	.795E-02	.791E-02	.775E-02	.739E-02	.720E-02	.702E-02
8.90	.791E-02	.787E-02	.783E-02	.779E-02	.775E-02	.771E-02	.756E-02	.721E-02	.702E-02	.685E-02
8.91	.771E-02	.767E-02	.764E-02	.760E-02	.756E-02	.752E-02	.737E-02	.703E-02	.684E-02	.667E-02
8.92	.752E-02	.748E-02	.745E-02	.741E-02	.737E-02	.733E-02	.718E-02	.685E-02	.667E-02	.651E-02
8.93	.733E-02	.730E-02	.726E-02	.722E-02	.719E-02	.715E-02	.700E-02	.668E-02	.650E-02	.634E-02
8.94	.715E-02	.712E-02	.708E-02	.704E-02	.701 -02	.697E-02	.683E-02	.651E-02	.634E-02	.618E-02
8.95	.697E-02	.694E-02	.690E-02	.687E-02	.683E-02	.680E-02	.666E-02	.635E-02	.618E-02	.602E-02
8.96	.680E-02	.677E-02	.673E-02	.670E-02	.666E-02	.663E-02	.649E-02	.619E-02	.602E-02	.587E-02
8.97	.663E-02	.660E-02	.656E-02	.653E-02	.650E-02	.646E-02	.633E-02	.603E-02	.587E-02	.572E-02
8.98	.646E-02	.643E-02	.640E-02	.636E-02	.633E-02	.630E-02	.617E-02	.588E-02	.572E-02	.557E-02
8.99	.630E-02	.627E-02	.624E-02	.620E-02	.617E-02	.614E-02	.601E-02	.573E-02	.557E-02	.543E-02
9.00	.614E-02	.611E-02	.608E-02	.605E-02	.602E-02	.599E-02	.586E-02	.558E-02	.543E-02	.529E-02

TABLE FOR E= 0.340E 01

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8.01	.655E-01	.652E-01	.649E-01	.646E-01	.643E-01	.640E-01	.629E-01	.604E-01	.591E-01	.578E-01
8.02	.640E-01	.637E-01	.634E-01	.631E-01	.628E-01	.626E-01	.615E-01	.590E-01	.577E-01	.565E-01
8.03	.625E-01	.622E-01	.619E-01	.617E-01	.614E-01	.611E-01	.601E-01	.577E-01	.564E-01	.552E-01
8.04	.610E-01	.608E-01	.605E-01	.602E-01	.600E-01	.597E-01	.587E-01	.563E-01	.551E-01	.539E-01
8.05	.596E-01	.594E-01	.591E-01	.589E-01	.586E-01	.583E-01	.573E-01	.550E-01	.538E-01	.527E-01
8.06	.583E-01	.580E-01	.578E-01	.575E-01	.572E-01	.570E-01	.560E-01	.538E-01	.525E-01	.515E-01
8.07	.569E-01	.567E-01	.564E-01	.562E-01	.559E-01	.557E-01	.547E-01	.525E-01	.513E-01	.503E-01
8.08	.556E-01	.554E-01	.551E-01	.549E-01	.546E-01	.544E-01	.534E-01	.513E-01	.501E-01	.491E-01
8.09	.543E-01	.541E-01	.538E-01	.536E-01	.534E-01	.531E-01	.522E-01	.501E-01	.490E-01	.480E-01
8.10	.531E-01	.529E-01	.526E-01	.524E-01	.521E-01	.519E-01	.510E-01	.490E-01	.478E-01	.468E-01
8.11	.518E-01	.516E-01	.514E-01	.512E-01	.509E-01	.507E-01	.498E-01	.478E-01	.467E-01	.458E-01
8.12	.506E-01	.504E-01	.502E-01	.500E-01	.498E-01	.495E-01	.487E-01	.467E-01	.456E-01	.447E-01
8.13	.495E-01	.493E-01	.491E-01	.488E-01	.486E-01	.484E-01	.475E-01	.456E-01	.446E-01	.437E-01
8.14	.483E-01	.481E-01	.479E-01	.477E-01	.475E-01	.473E-01	.464E-01	.446E-01	.436E-01	.426E-01
8.15	.472E-01	.470E-01	.468E-01	.466E-01	.464E-01	.462E-01	.454E-01	.435E-01	.425E-01	.417E-01
8.16	.461E-01	.459E-01	.457E-01	.455E-01	.453E-01	.451E-01	.443E-01	.425E-01	.416E-01	.407E-01
8.17	.450E-01	.449E-01	.447E-01	.445E-01	.443E-01	.441E-01	.433E-01	.415E-01	.406E-01	.397E-01
8.18	.440E-01	.438E-01	.436E-01	.434E-01	.432E-01	.430E-01	.423E-01	.406E-01	.396E-01	.388E-01
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8.20	.420E-01	.418E-01	.416E-01	.414E-01	.413E-01	.411E-01	.403E-01	.387E-01	.378E-01	.370E-01
8.21	.410E-01	.408E-01	.407E-01	.405E-01	.403E-01	.401E-01	.394E-01	.378E-01	.369E-01	.362E-01
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8.25	.373E-01	.372E-01	.370E-01	.368E-01	.367E-01	.365E-01	.359E-01	.344E-01	.336E-01	.329E-01
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8.27	.356E-01	.355E-01	.353E-01	.351E-01	.350E-01	.348E-01	.342E-01	.328E-01	.321E-01	.314E-01
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8.29	.340E-01	.338E-01	.337E-01	.335E-01	.334E-01	.332E-01	.326E-01	.313E-01	.306E-01	.299E-01
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8.33	.309E-01	.308E-01	.306E-01	.305E-01	.304E-01	.302E-01	.297E-01	.285E-01	.278E-01	.272E-01
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8.48	.217E-01	.216E-01	.215E-01	.214E-01	.213E-01	.212E-01	.208E-01	.199E-01	.194E-01	.190E-01
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8.51	.202E-01	.201E-01	.200E-01	.199E-01	.198E-01	.197E-01	.193E-01	.185E-01	.181E-01	.177E-01
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8.64	.148E-01	.147E-01	.146E-01	.146E-01	.145E-01	.144E-01	.141E-01	.135E-01	.132E-01	.129E-01
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8.66	.141E-01	.140E-01	.139E-01	.139E-01	.138E-01	.137E-01	.135E-01	.129E-01	.126E-01	.123E-01
8.67	.137E-01	.137E-01	.136E-01	.135E-01	.135E-01	.134E-01	.132E-01	.126E-01	.123E-01	.120E-01
8.68	.134E-01	.133E-01	.133E-01	.132E-01	.131E-01	.131E-01	.128E-01	.123E-01	.120E-01	.117E-01
8.69	.131E-01	.130E-01	.130E-01	.129E-01	.128E-01	.128E-01	.125E-01	.120E-01	.117E-01	.114E-01
8.70	.128E-01	.127E-01	.126E-01	.126E-01	.125E-01	.125E-01	.122E-01	.117E-01	.114E-01	.111E-01
8.71	.125E-01	.124E-01	.123E-01	.123E-01	.122E-01	.122E-01	.119E-01	.114E-01	.111E-01	.109E-01
8.72	.122E-01	.121E-01	.120E-01	.120E-01	.119E-01	.119E-01	.116E-01	.111E-01	.109E-01	.106E-01
8.73	.119E-01	.118E-01	.117E-01	.117E-01	.116E-01	.116E-01	.114E-01	.109E-01	.106E-01	.103E-01
8.74	.116E-01	.115E-01	.115E-01	.114E-01	.114E-01	.113E-01	.111E-01	.106E-01	.103E-01	.101E-01
8.75	.113E-01	.112E-01	.112E-01	.111E-01	.111E-01	.110E-01	.108E-01	.103E-01	.101E-01	.984E-02
8.76	.110E-01	.110E-01	.109E-01	.109E-01	.108E-01	.108E-01	.106E-01	.101E-01	.983E-02	.960E-02
8.77	.108E-01	.107E-01	.107E-01	.106E-01	.105E-01	.105E-01	.103E-01	.984E-02	.959E-02	.937E-02
8.78	.105E-01	.104E-01	.104E-01	.103E-01	.103E-01	.102E-01	.100E-01	.960E-02	.935E-02	.913E-02
8.79	.102E-01	.102E-01	.101E-01	.101E-01	.100E-01	.999E-02	.980E-02	.936E-02	.912E-02	.891E-02
8.80	.999E-02	.994E-02	.989E-02	.985E-02	.980E-02	.975E-02	.956E-02	.913E-02	.890E-02	.869E-02
8.81	.975E-02	.970E-02	.965E-02	.961E-02	.956E-02	.951E-02	.932E-02	.891E-02	.868E-02	.847E-02
8.82	.951E-02	.946E-02	.942E-02	.937E-02	.932E-02	.928E-02	.910E-02	.869E-02	.846E-02	.826E-02
8.83	.928E-02	.923E-02	.919E-02	.914E-02	.910E-02	.905E-02	.887E-02	.847E-02	.825E-02	.806E-02
8.84	.905E-02	.901E-02	.896E-02	.892E-02	.887E-02	.883E-02	.865E-02	.826E-02	.805E-02	.786E-02
8.85	.883E-02	.879E-02	.874E-02	.870E-02	.866E-02	.861E-02	.844E-02	.806E-02	.785E-02	.766E-02
8.86	.861E-02	.857E-02	.853E-02	.848E-02	.844E-02	.840E-02	.823E-02	.786E-02	.765E-02	.747E-02
8.87	.840E-02	.836E-02	.832E-02	.828E-02	.823E-02	.819E-02	.803E-02	.766E-02	.746E-02	.728E-02
8.88	.819E-02	.815E-02	.811E-02	.807E-02	.803E-02	.799E-02	.783E-02	.747E-02	.728E-02	.710E-02
8.89	.799E-02	.795E-02	.791E-02	.787E-02	.783E-02	.779E-02	.764E-02	.729E-02	.709E-02	.692E-02
8.90	.779E-02	.775E-02	.772E-02	.768E-02	.764E-02	.760E-02	.745E-02	.710E-02	.692E-02	.675E-02
8.91	.760E-02	.756E-02	.752E-02	.749E-02	.745E-02	.741E-02	.726E-02	.693E-02	.674E-02	.658E-02
8.92	.741E-02	.738E-02	.734E-02	.730E-02	.726E-02	.723E-02	.708E-02	.675E-02	.657E-02	.641E-02
8.93	.723E-02	.719E-02	.716E-02	.712E-02	.708E-02	.705E-02	.690E-02	.658E-02	.641E-02	.625E-02
8.94	.705E-02	.701E-02	.698E-02	.694E-02	.691E-02	.687E-02	.673E-02	.642E-02	.624E-02	.609E-02
8.95	.687E-02	.684E-02	.680E-02	.677E-02	.673E-02	.670E-02	.656E-02	.625E-02	.609E-02	.594E-02
8.96	.670E-02	.667E-02	.663E-02	.660E-02	.657E-02	.653E-02	.640E-02	.610E-02	.593E-02	.578E-02
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8.98	.637E-02	.634E-02	.631E-02	.627E-02	.624E-02	.621E-02	.608E-02	.579E-02	.563E-02	.549E-02
8.99	.621E-02	.618E-02	.615E-02	.611E-02	.608E-02	.605E-02	.592E-02	.564E-02	.549E-02	.535E-02
9.00	.605E-02	.602E-02	.599E-02	.596E-02	.593E-02	.590E-02	.577E-02	.550E-02	.535E-02	.521E-02

TABLE FOR E= 0.33530E 01

Y=	.940E 00	.938E 00	.936E 00	.934E 00	.932E 00	.930E 00	.922E 00	.904E 00	.894E 00	.885E 00
8.00	.660E-01	.657E-01	.655E-01	.652E-01	.649E-01	.646E-01	.635E-01	.609E-01	.596E-01	.583E-01
8.01	.645E-01	.642E-01	.639E-01	.637E-01	.634E-01	.631E-01	.620E-01	.595E-01	.582E-01	.570E-01
8.02	.630E-01	.627E-01	.625E-01	.622E-01	.619E-01	.617E-01	.606E-01	.582E-01	.568E-01	.557E-01
8.03	.616E-01	.613E-01	.610E-01	.608E-01	.605E-01	.602E-01	.592E-01	.568E-01	.555E-01	.544E-01
8.04	.601E-01	.599E-01	.596E-01	.594E-01	.591E-01	.588E-01	.578E-01	.555E-01	.542E-01	.531E-01
8.05	.588E-01	.585E-01	.582E-01	.580E-01	.577E-01	.575E-01	.565E-01	.542E-01	.530E-01	.519E-01
8.06	.574E-01	.572E-01	.569E-01	.567E-01	.564E-01	.562E-01	.552E-01	.530E-01	.518E-01	.507E-01
8.07	.561E-01	.559E-01	.556E-01	.553E-01	.551E-01	.549E-01	.539E-01	.517E-01	.506E-01	.495E-01
8.08	.548E-01	.545E-01	.543E-01	.541E-01	.538E-01	.536E-01	.526E-01	.505E-01	.494E-01	.484E-01
8.09	.535E-01	.533E-01	.531E-01	.528E-01	.526E-01	.524E-01	.514E-01	.494E-01	.483E-01	.473E-01
8.10	.523E-01	.521E-01	.518E-01	.516E-01	.514E-01	.511E-01	.502E-01	.482E-01	.471E-01	.462E-01
8.11	.511E-01	.509E-01	.506E-01	.504E-01	.502E-01	.500E-01	.491E-01	.471E-01	.460E-01	.451E-01
8.12	.499E-01	.497E-01	.495E-01	.492E-01	.490E-01	.488E-01	.479E-01	.460E-01	.450E-01	.440E-01
8.13	.487E-01	.485E-01	.483E-01	.481E-01	.479E-01	.477E-01	.468E-01	.450E-01	.439E-01	.430E-01
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8.15	.465E-01	.463E-01	.461E-01	.459E-01	.457E-01	.455E-01	.447E-01	.429E-01	.419E-01	.410E-01
8.16	.454E-01	.452E-01	.450E-01	.448E-01	.446E-01	.444E-01	.437E-01	.419E-01	.409E-01	.401E-01
8.17	.444E-01	.442E-01	.440E-01	.438E-01	.436E-01	.434E-01	.426E-01	.409E-01	.400E-01	.392E-01
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8.22	.395E-01	.393E-01	.391E-01	.390E-01	.388E-01	.386E-01	.379E-01	.364E-01	.356E-01	.348E-01
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8.24	.377E-01	.375E-01	.373E-01	.372E-01	.370E-01	.368E-01	.362E-01	.347E-01	.339E-01	.332E-01
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8.26	.359E-01	.358E-01	.356E-01	.355E-01	.353E-01	.351E-01	.345E-01	.331E-01	.323E-01	.317E-01
8.27	.351E-01	.349E-01	.348E-01	.346E-01	.345E-01	.343E-01	.337E-01	.323E-01	.316E-01	.309E-01
8.28	.343E-01	.341E-01	.340E-01	.338E-01	.337E-01	.335E-01	.329E-01	.316E-01	.309E-01	.302E-01
8.29	.335E-01	.333E-01	.332E-01	.330E-01	.329E-01	.327E-01	.322E-01	.308E-01	.301E-01	.295E-01
8.30	.327E-01	.326E-01	.324E-01	.323E-01	.321E-01	.320E-01	.314E-01	.301E-01	.294E-01	.288E-01
8.31	.319E-01	.318E-01	.317E-01	.315E-01	.314E-01	.312E-01	.307E-01	.294E-01	.287E-01	.281E-01
8.32	.312E-01	.311E-01	.309E-01	.308E-01	.306E-01	.305E-01	.300E-01	.287E-01	.281E-01	.275E-01
8.33	.305E-01	.303E-01	.302E-01	.301E-01	.299E-01	.298E-01	.293E-01	.281E-01	.274E-01	.268E-01
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8.35	.291E-01	.289E-01	.288E-01	.287E-01	.285E-01	.284E-01	.279E-01	.268E-01	.261E-01	.256E-01
8.36	.284E-01	.283E-01	.281E-01	.280E-01	.279E-01	.278E-01	.273E-01	.261E-01	.255E-01	.250E-01
8.37	.277E-01	.276E-01	.275E-01	.274E-01	.272E-01	.271E-01	.266E-01	.255E-01	.249E-01	.244E-01
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8.39	.264E-01	.263E-01	.262E-01	.261E-01	.260E-01	.258E-01	.254E-01	.243E-01	.238E-01	.233E-01
8.40	.258E-01	.257E-01	.256E-01	.255E-01	.254E-01	.252E-01	.248E-01	.238E-01	.232E-01	.227E-01
8.41	.252E-01	.251E-01	.250E-01	.249E-01	.248E-01	.247E-01	.242E-01	.232E-01	.227E-01	.222E-01
8.42	.246E-01	.245E-01	.244E-01	.243E-01	.242E-01	.241E-01	.236E-01	.227E-01	.221E-01	.216E-01
8.43	.240E-01	.239E-01	.238E-01	.237E-01	.236E-01	.235E-01	.231E-01	.221E-01	.216E-01	.211E-01
8.44	.235E-01	.234E-01	.233E-01	.232E-01	.231E-01	.230E-01	.225E-01	.216E-01	.211E-01	.206E-01
8.45	.229E-01	.228E-01	.227E-01	.226E-01	.225E-01	.224E-01	.220E-01	.211E-01	.206E-01	.201E-01
8.46	.224E-01	.223E-01	.222E-01	.221E-01	.220E-01	.219E-01	.215E-01	.206E-01	.201E-01	.197E-01
8.47	.219E-01	.218E-01	.217E-01	.216E-01	.215E-01	.214E-01	.210E-01	.201E-01	.196E-01	.192E-01
8.48	.214E-01	.213E-01	.212E-01	.211E-01	.210E-01	.209E-01	.205E-01	.196E-01	.192E-01	.187E-01
8.49	.208E-01	.208E-01	.207E-01	.206E-01	.205E-01	.204E-01	.200E-01	.192E-01	.187E-01	.183E-01
8.50	.204E-01	.203E-01	.202E-01	.201E-01	.200E-01	.199E-01	.195E-01	.187E-01	.183E-01	.179E-01
8.51	.199E-01	.198E-01	.197E-01	.196E-01	.195E-01	.194E-01	.191E-01	.183E-01	.178E-01	.174E-01
8.52	.194E-01	.193E-01	.192E-01	.191E-01	.191E-01	.190E-01	.186E-01	.178E-01	.174E-01	.170E-01
8.53	.189E-01	.189E-01	.188E-01	.187E-01	.186E-01	.185E-01	.182E-01	.174E-01	.170E-01	.166E-01
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8.57	.172E-01	.171E-01	.171E-01	.170E-01	.169E-01	.168E-01	.165E-01	.158E-01	.154E-01	.151E-01
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8.60	.160E-01	.159E-01	.159E-01	.158E-01	.157E-01	.156E-01	.154E-01	.147E-01	.143E-01	.140E-01
8.61	.156E-01	.156E-01	.155E-01	.154E-01	.153E-01	.153E-01	.150E-01	.143E-01	.140E-01	.137E-01
8.62	.153E-01	.152E-01	.151E-01	.151E-01	.150E-01	.149E-01	.146E-01	.140E-01	.137E-01	.134E-01
8.63	.149E-01	.148E-01	.148E-01	.147E-01	.146E-01	.146E-01	.143E-01	.137E-01	.133E-01	.130E-01
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8.67	.135E-01	.135E-01	.134E-01	.133E-01	.133E-01	.132E-01	.130E-01	.124E-01	.121E-01	.118E-01
8.68	.132E-01	.131E-01	.131E-01	.130E-01	.130E-01	.129E-01	.126E-01	.121E-01	.118E-01	.115E-01
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8.74	.114E-01	.114E-01	.113E-01	.112E-01	.112E-01	.111E-01	.109E-01	.104E-01	.102E-01	.994E-02
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8.81	.960E-02	.956E-02	.951E-02	.946E-02	.942E-02	.937E-02	.919E-02	.878E-02	.855E-02	.835E-02
8.82	.937E-02	.932E-02	.928E-02	.923E-02	.919E-02	.914E-02	.896E-02	.856E-02	.834E-02	.814E-02
8.83	.914E-02	.910E-02	.905E-02	.901E-02	.896E-02	.892E-02	.874E-02	.835E-02	.813E-02	.794E-02
8.84	.892E-02	.887E-02	.883E-02	.879E-02	.874E-02	.870E-02	.853E-02	.814E-02	.793E-02	.774E-02
8.85	.870E-02	.866E-02	.861E-02	.857E-02	.853E-02	.849E-02	.832E-02	.794E-02	.773E-02	.755E-02
8.86	.849E-02	.844E-02	.840E-02	.836E-02	.832E-02	.828E-02	.811E-02	.774E-02	.754E-02	.736E-02
8.87	.828E-02	.824E-02	.819E-02	.815E-02	.811E-02	.807E-02	.791E-02	.755E-02	.735E-02	.718E-02
8.88	.807E-02	.803E-02	.799E-02	.795E-02	.791E-02	.787E-02	.771E-02	.736E-02	.717E-02	.700E-02
8.89	.787E-02	.783E-02	.780E-02	.776E-02	.772E-02	.768E-02	.752E-02	.718E-02	.699E-02	.682E-02
8.90	.768E-02	.764E-02	.760E-02	.756E-02	.753E-02	.749E-02	.734E-02	.700E-02	.681E-02	.665E-02
8.91	.749E-02	.745E-02	.741E-02	.738E-02	.734E-02	.730E-02	.715E-02	.682E-02	.664E-02	.648E-02
8.92	.730E-02	.727E-02	.723E-02	.719E-02	.716E-02	.712E-02	.698E-02	.665E-02	.648E-02	.632E-02
8.93	.712E-02	.709E-02	.705E-02	.701E-02	.698E-02	.694E-02	.680E-02	.649E-02	.631E-02	.616E-02
8.94	.695E-02	.691E-02	.687E-02	.684E-02	.681E-02	.677E-02	.663E-02	.632E-02	.615E-02	.600E-02
8.95	.677E-02	.674E-02	.670E-02	.667E-02	.664E-02	.660E-02	.646E-02	.616E-02	.600E-02	.585E-02
8.96	.660E-02	.657E-02	.654E-02	.650E-02	.647E-02	.644E-02	.630E-02	.601E-02	.584E-02	.570E-02
8.97	.644E-02	.641E-02	.637E-02	.634E-02	.631E-02	.627E-02	.614E-02	.585E-02	.570E-02	.555E-02
8.98	.628E-02	.624E-02	.621E-02	.618E-02	.615E-02	.612E-02	.599E-02	.571E-02	.555E-02	.541E-02
8.99	.612E-02	.609E-02	.606E-02	.602E-02	.599E-02	.596E-02	.584E-02	.556E-02	.541E-02	.527E-02
9.00	.597E-02	.593E-02	.590E-02	.587E-02	.584E-02	.581E-02	.569E-02	.542E-02	.527E-02	.514E-02

TABLE EQB E= 6.33333E-01

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8.00	.650E-01	.648E-01	.645E-01	.642E-01	.639E-01	.636E-01	.625E-01	.600E-01	.587E-01	.575E-01
8.01	.635E-01	.633E-01	.630E-01	.627E-01	.624E-01	.622E-01	.611E-01	.586E-01	.573E-01	.561E-01
8.02	.621E-01	.618E-01	.615E-01	.613E-01	.610E-01	.607E-01	.597E-01	.573E-01	.560E-01	.548E-01
8.03	.606E-01	.604E-01	.601E-01	.599E-01	.596E-01	.593E-01	.583E-01	.560E-01	.547E-01	.536E-01
8.04	.592E-01	.590E-01	.587E-01	.585E-01	.582E-01	.580E-01	.569E-01	.547E-01	.534E-01	.523E-01
8.05	.579E-01	.576E-01	.574E-01	.571E-01	.569E-01	.566E-01	.556E-01	.534E-01	.522E-01	.511E-01
8.06	.565E-01	.563E-01	.561E-01	.558E-01	.556E-01	.553E-01	.543E-01	.522E-01	.510E-01	.499E-01
8.07	.552E-01	.550E-01	.548E-01	.545E-01	.543E-01	.540E-01	.531E-01	.510E-01	.498E-01	.488E-01
8.08	.540E-01	.537E-01	.535E-01	.533E-01	.530E-01	.528E-01	.519E-01	.498E-01	.487E-01	.477E-01
8.09	.527E-01	.525E-01	.523E-01	.520E-01	.518E-01	.516E-01	.507E-01	.486E-01	.475E-01	.465E-01
8.10	.515E-01	.513E-01	.511E-01	.508E-01	.506E-01	.504E-01	.495E-01	.475E-01	.464E-01	.455E-01
8.11	.503E-01	.501E-01	.499E-01	.497E-01	.494E-01	.492E-01	.483E-01	.464E-01	.454E-01	.444E-01
8.12	.492E-01	.489E-01	.487E-01	.485E-01	.483E-01	.481E-01	.472E-01	.453E-01	.443E-01	.434E-01
8.13	.480E-01	.478E-01	.476E-01	.474E-01	.472E-01	.470E-01	.461E-01	.443E-01	.433E-01	.424E-01
8.14	.469E-01	.467E-01	.465E-01	.463E-01	.461E-01	.459E-01	.451E-01	.433E-01	.423E-01	.414E-01
8.15	.458E-01	.456E-01	.454E-01	.452E-01	.450E-01	.448E-01	.440E-01	.423E-01	.413E-01	.404E-01
8.16	.448E-01	.446E-01	.444E-01	.442E-01	.440E-01	.438E-01	.430E-01	.413E-01	.403E-01	.395E-01
8.17	.437E-01	.435E-01	.433E-01	.431E-01	.430E-01	.428E-01	.420E-01	.403E-01	.394E-01	.386E-01
8.18	.427E-01	.425E-01	.423E-01	.421E-01	.420E-01	.418E-01	.410E-01	.394E-01	.385E-01	.377E-01
8.19	.417E-01	.415E-01	.414E-01	.412E-01	.410E-01	.408E-01	.401E-01	.385E-01	.376E-01	.368E-01
8.20	.408E-01	.406E-01	.404E-01	.402E-01	.400E-01	.399E-01	.392E-01	.376E-01	.367E-01	.359E-01
8.21	.398E-01	.396E-01	.395E-01	.393E-01	.391E-01	.389E-01	.382E-01	.367E-01	.359E-01	.351E-01
8.22	.389E-01	.387E-01	.385E-01	.384E-01	.382E-01	.380E-01	.374E-01	.358E-01	.350E-01	.343E-01
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8.24	.371E-01	.369E-01	.368E-01	.366E-01	.364E-01	.363E-01	.356E-01	.342E-01	.334E-01	.327E-01
8.25	.362E-01	.361E-01	.359E-01	.358E-01	.356E-01	.354E-01	.348E-01	.334E-01	.326E-01	.319E-01
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8.27	.346E-01	.344E-01	.343E-01	.341E-01	.340E-01	.338E-01	.332E-01	.319E-01	.311E-01	.305E-01
8.28	.338E-01	.336E-01	.335E-01	.333E-01	.332E-01	.330E-01	.324E-01	.311E-01	.304E-01	.298E-01
8.29	.330E-01	.328E-01	.327E-01	.325E-01	.324E-01	.323E-01	.317E-01	.304E-01	.297E-01	.291E-01
8.30	.322E-01	.321E-01	.319E-01	.318E-01	.316E-01	.315E-01	.309E-01	.297E-01	.290E-01	.284E-01
8.31	.315E-01	.313E-01	.312E-01	.310E-01	.309E-01	.308E-01	.302E-01	.290E-01	.283E-01	.277E-01
8.32	.307E-01	.306E-01	.305E-01	.303E-01	.302E-01	.301E-01	.295E-01	.283E-01	.276E-01	.271E-01
8.33	.300E-01	.299E-01	.297E-01	.296E-01	.295E-01	.293E-01	.288E-01	.276E-01	.270E-01	.264E-01
8.34	.293E-01	.292E-01	.291E-01	.289E-01	.288E-01	.287E-01	.281E-01	.270E-01	.264E-01	.258E-01
8.35	.286E-01	.285E-01	.284E-01	.282E-01	.281E-01	.280E-01	.275E-01	.264E-01	.257E-01	.252E-01
8.36	.280E-01	.278E-01	.277E-01	.276E-01	.275E-01	.273E-01	.268E-01	.257E-01	.251E-01	.246E-01
8.37	.273E-01	.272E-01	.271E-01	.269E-01	.268E-01	.267E-01	.262E-01	.251E-01	.246E-01	.240E-01
8.38	.267E-01	.266E-01	.264E-01	.263E-01	.262E-01	.261E-01	.256E-01	.246E-01	.240E-01	.235E-01
8.39	.260E-01	.259E-01	.258E-01	.257E-01	.256E-01	.255E-01	.250E-01	.240E-01	.234E-01	.229E-01
8.40	.254E-01	.253E-01	.252E-01	.251E-01	.250E-01	.249E-01	.244E-01	.234E-01	.229E-01	.224E-01
8.41	.248E-01	.247E-01	.246E-01	.245E-01	.244E-01	.243E-01	.238E-01	.229E-01	.223E-01	.218E-01
8.42	.243E-01	.241E-01	.240E-01	.239E-01	.238E-01	.237E-01	.233E-01	.223E-01	.218E-01	.213E-01
8.43	.237E-01	.236E-01	.235E-01	.234E-01	.233E-01	.232E-01	.227E-01	.218E-01	.213E-01	.208E-01
8.44	.231E-01	.230E-01	.229E-01	.228E-01	.227E-01	.226E-01	.222E-01	.213E-01	.208E-01	.203E-01
8.45	.226E-01	.225E-01	.224E-01	.223E-01	.222E-01	.221E-01	.217E-01	.208E-01	.203E-01	.198E-01
8.46	.221E-01	.220E-01	.219E-01	.218E-01	.217E-01	.216E-01	.212E-01	.203E-01	.198E-01	.194E-01
8.47	.215E-01	.214E-01	.213E-01	.212E-01	.211E-01	.211E-01	.207E-01	.198E-01	.193E-01	.189E-01
8.48	.210E-01	.209E-01	.208E-01	.207E-01	.207E-01	.206E-01	.202E-01	.193E-01	.189E-01	.185E-01
8.49	.205E-01	.204E-01	.204E-01	.203E-01	.202E-01	.201E-01	.197E-01	.189E-01	.184E-01	.180E-01
8.50	.201E-01	.200E-01	.199E-01	.198E-01	.197E-01	.196E-01	.192E-01	.184E-01	.180E-01	.176E-01
8.51	.196E-01	.195E-01	.194E-01	.193E-01	.192E-01	.191E-01	.188E-01	.180E-01	.176E-01	.172E-01
8.52	.191E-01	.190E-01	.189E-01	.189E-01	.188E-01	.187E-01	.183E-01	.176E-01	.171E-01	.168E-01
8.53	.187E-01	.186E-01	.185E-01	.184E-01	.183E-01	.182E-01	.179E-01	.171E-01	.167E-01	.164E-01
8.54	.182E-01	.181E-01	.181E-01	.180E-01	.179E-01	.178E-01	.175E-01	.167E-01	.163E-01	.160E-01
8.55	.178E-01	.177E-01	.176E-01	.175E-01	.175E-01	.174E-01	.171E-01	.163E-01	.159E-01	.156E-01
8.56	.174E-01	.173E-01	.172E-01	.171E-01	.171E-01	.170E-01	.167E-01	.160E-01	.156E-01	.152E-01
8.57	.170E-01	.169E-01	.168E-01	.167E-01	.166E-01	.166E-01	.163E-01	.156E-01	.152E-01	.149E-01
8.58	.166E-01	.165E-01	.164E-01	.163E-01	.163E-01	.162E-01	.159E-01	.152E-01	.148E-01	.145E-01
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8.60	.158E-01	.157E-01	.156E-01	.156E-01	.155E-01	.154E-01	.151E-01	.145E-01	.141E-01	.138E-01
8.61	.154E-01	.153E-01	.153E-01	.152E-01	.151E-01	.150E-01	.148E-01	.141E-01	.138E-01	.135E-01
8.62	.150E-01	.150E-01	.149E-01	.148E-01	.148E-01	.147E-01	.144E-01	.138E-01	.135E-01	.132E-01
8.63	.147E-01	.146E-01	.145E-01	.145E-01	.144E-01	.143E-01	.141E-01	.135E-01	.131E-01	.128E-01
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8.67	.133E-01	.133E-01	.132E-01	.131E-01	.131E-01	.130E-01	.128E-01	.122E-01	.119E-01	.116E-01
8.68	.130E-01	.129E-01	.129E-01	.128E-01	.128E-01	.127E-01	.125E-01	.119E-01	.116E-01	.114E-01
8.69	.127E-01	.126E-01	.126E-01	.125E-01	.125E-01	.124E-01	.122E-01	.116E-01	.113E-01	.111E-01
8.70	.124E-01	.123E-01	.123E-01	.122E-01	.122E-01	.121E-01	.119E-01	.113E-01	.111E-01	.108E-01
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8.72	.118E-01	.117E-01	.117E-01	.116E-01	.116E-01	.115E-01	.113E-01	.108E-01	.105E-01	.103E-01
8.73	.115E-01	.115E-01	.114E-01	.113E-01	.113E-01	.112E-01	.110E-01	.105E-01	.103E-01	.100E-01
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8.75	.110E-01	.109E-01	.109E-01	.108E-01	.108E-01	.107E-01	.105E-01	.100E-01	.978E-02	.955E-02
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8.77	.104E-01	.104E-01	.103E-01	.103E-01	.102E-01	.102E-01	.999E-02	.955E-02	.931E-02	.909E-02
8.78	.102E-01	.101E-01	.101E-01	.100E-01	.999E-02	.994E-02	.975E-02	.931E-02	.908E-02	.887E-02
8.79	.994E-02	.989E-02	.984E-02	.979E-02	.975E-02	.970E-02	.951E-02	.909E-02	.885E-02	.865E-02
8.80	.970E-02	.965E-02	.960E-02	.956E-02	.951E-02	.946E-02	.928E-02	.886E-02	.864E-02	.843E-02
8.81	.946E-02	.941E-02	.937E-02	.932E-02	.928E-02	.923E-02	.905E-02	.864E-02	.842E-02	.822E-02
8.82	.923E-02	.918E-02	.914E-02	.910E-02	.905E-02	.901E-02	.883E-02	.843E-02	.821E-02	.802E-02
8.83	.900E-02	.896E-02	.892E-02	.887E-02	.883E-02	.878E-02	.861E-02	.822E-02	.801E-02	.782E-02
8.84	.878E-02	.874E-02	.870E-02	.866E-02	.861E-02	.857E-02	.840E-02	.802E-02	.781E-02	.763E-02
8.85	.857E-02	.853E-02	.848E-02	.844E-02	.840E-02	.836E-02	.819E-02	.782E-02	.762E-02	.744E-02
8.86	.836E-02	.832E-02	.828E-02	.824E-02	.819E-02	.815E-02	.799E-02	.763E-02	.743E-02	.725E-02
8.87	.815E-02	.811E-02	.807E-02	.803E-02	.799E-02	.795E-02	.779E-02	.744E-02	.724E-02	.707E-02
8.88	.795E-02	.791E-02	.787E-02	.783E-02	.779E-02	.776E-02	.760E-02	.725E-02	.706E-02	.689E-02
8.89	.776E-02	.772E-02	.768E-02	.764E-02	.760E-02	.756E-02	.741E-02	.707E-02	.689E-02	.672E-02
8.90	.756E-02	.753E-02	.749E-02	.745E-02	.741E-02	.738E-02	.723E-02	.689E-02	.671E-02	.655E-02
8.91	.738E-02	.734E-02	.730E-02	.727E-02	.723E-02	.719E-02	.705E-02	.672E-02	.654E-02	.638E-02
8.92	.719E-02	.716E-02	.712E-02	.709E-02	.705E-02	.701E-02	.687E-02	.655E-02	.638E-02	.622E-02
8.93	.702E-02	.698E-02	.695E-02	.691E-02	.687E-02	.684E-02	.670E-02	.639E-02	.622E-02	.607E-02
8.94	.684E-02	.681E-02	.677E-02	.674E-02	.670E-02	.667E-02	.653E-02	.623E-02	.606E-02	.591E-02
8.95	.667E-02	.664E-02	.660E-02	.657E-02	.654E-02	.650E-02	.637E-02	.607E-02	.591E-02	.576E-02
8.96	.650E-02	.647E-02	.644E-02	.641E-02	.637E-02	.634E-02	.621E-02	.592E-02	.576E-02	.561E-02
8.97	.634E-02	.631E-02	.628E-02	.624E-02	.621E-02	.618E-02	.605E-02	.577E-02	.561E-02	.547E-02
8.98	.618E-02	.615E-02	.612E-02	.609E-02	.606E-02	.603E-02	.590E-02	.562E-02	.547E-02	.533E-02
8.99	.603E-02	.600E-02	.597E-02	.593E-02	.590E-02	.587E-02	.575E-02	.548E-02	.533E-02	.519E-02
9.00	.588E-02	.585E-02	.582E-02	.579E-02	.576E-02	.573E-02	.561E-02	.534E-02	.519E-02	.506E-02

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Y=	.940E 00	.938E 00	.936E 00	.934E 00	.932E 00	.930E 00	.922E 00	.904E 00	.894E 00	.885E 00
8.00	.641E-01	.638E-01	.635E-01	.632E-01	.629E-01	.627E-01	.616E-01	.591E-01	.578E-01	.566E-01
8.01	.626E-01	.623E-01	.620E-01	.618E-01	.615E-01	.612E-01	.601E-01	.578E-01	.565E-01	.553E-01
8.02	.611E-01	.609E-01	.606E-01	.603E-01	.601E-01	.598E-01	.588E-01	.564E-01	.551E-01	.540E-01
8.03	.597E-01	.595E-01	.592E-01	.589E-01	.587E-01	.584E-01	.574E-01	.551E-01	.539E-01	.528E-01
8.04	.584E-01	.581E-01	.578E-01	.576E-01	.573E-01	.571E-01	.561E-01	.538E-01	.526E-01	.515E-01
8.05	.570E-01	.568E-01	.565E-01	.563E-01	.560E-01	.558E-01	.548E-01	.526E-01	.514E-01	.503E-01
8.06	.557E-01	.554E-01	.552E-01	.550E-01	.547E-01	.545E-01	.535E-01	.514E-01	.502E-01	.492E-01
8.07	.544E-01	.542E-01	.539E-01	.537E-01	.535E-01	.532E-01	.523E-01	.502E-01	.491E-01	.480E-01
8.08	.532E-01	.529E-01	.527E-01	.525E-01	.522E-01	.520E-01	.511E-01	.490E-01	.479E-01	.469E-01
8.09	.519E-01	.517E-01	.515E-01	.512E-01	.510E-01	.508E-01	.499E-01	.479E-01	.468E-01	.458E-01
8.10	.507E-01	.505E-01	.503E-01	.501E-01	.498E-01	.496E-01	.487E-01	.468E-01	.457E-01	.448E-01
8.11	.496E-01	.493E-01	.491E-01	.489E-01	.487E-01	.485E-01	.476E-01	.457E-01	.447E-01	.437E-01
8.12	.484E-01	.482E-01	.480E-01	.478E-01	.476E-01	.474E-01	.465E-01	.447E-01	.436E-01	.427E-01
8.13	.473E-01	.471E-01	.469E-01	.467E-01	.465E-01	.463E-01	.454E-01	.436E-01	.426E-01	.417E-01
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8.16	.441E-01	.439E-01	.437E-01	.435E-01	.433E-01	.431E-01	.424E-01	.407E-01	.397E-01	.389E-01
8.17	.431E-01	.429E-01	.427E-01	.425E-01	.423E-01	.421E-01	.414E-01	.397E-01	.388E-01	.380E-01
8.18	.421E-01	.419E-01	.417E-01	.415E-01	.413E-01	.411E-01	.404E-01	.388E-01	.379E-01	.371E-01
8.19	.411E-01	.409E-01	.407E-01	.405E-01	.404E-01	.402E-01	.395E-01	.379E-01	.370E-01	.362E-01
8.20	.401E-01	.400E-01	.398E-01	.396E-01	.394E-01	.393E-01	.386E-01	.370E-01	.362E-01	.354E-01
8.21	.392E-01	.390E-01	.389E-01	.387E-01	.385E-01	.383E-01	.377E-01	.361E-01	.353E-01	.346E-01
8.22	.383E-01	.381E-01	.380E-01	.378E-01	.376E-01	.375E-01	.368E-01	.353E-01	.345E-01	.338E-01
8.23	.374E-01	.372E-01	.371E-01	.369E-01	.367E-01	.366E-01	.359E-01	.345E-01	.337E-01	.330E-01
8.24	.365E-01	.364E-01	.362E-01	.361E-01	.359E-01	.357E-01	.351E-01	.337E-01	.329E-01	.322E-01
8.25	.357E-01	.355E-01	.354E-01	.352E-01	.351E-01	.349E-01	.343E-01	.329E-01	.321E-01	.315E-01
8.26	.349E-01	.347E-01	.346E-01	.344E-01	.342E-01	.341E-01	.335E-01	.321E-01	.314E-01	.307E-01
8.27	.341E-01	.339E-01	.337E-01	.336E-01	.334E-01	.333E-01	.327E-01	.314E-01	.307E-01	.300E-01
8.28	.333E-01	.331E-01	.330E-01	.328E-01	.327E-01	.325E-01	.319E-01	.306E-01	.299E-01	.293E-01
8.29	.325E-01	.323E-01	.322E-01	.321E-01	.319E-01	.318E-01	.312E-01	.299E-01	.292E-01	.286E-01
8.30	.317E-01	.316E-01	.314E-01	.313E-01	.312E-01	.310E-01	.305E-01	.292E-01	.286E-01	.279E-01
8.31	.310E-01	.309E-01	.307E-01	.306E-01	.304E-01	.303E-01	.298E-01	.285E-01	.279E-01	.273E-01
8.32	.303E-01	.301E-01	.300E-01	.299E-01	.297E-01	.296E-01	.291E-01	.279E-01	.272E-01	.267E-01
8.33	.296E-01	.294E-01	.293E-01	.292E-01	.290E-01	.289E-01	.284E-01	.272E-01	.266E-01	.260E-01
8.34	.289E-01	.287E-01	.286E-01	.285E-01	.284E-01	.282E-01	.277E-01	.266E-01	.260E-01	.254E-01
8.35	.282E-01	.281E-01	.279E-01	.278E-01	.277E-01	.276E-01	.271E-01	.260E-01	.254E-01	.248E-01
8.36	.275E-01	.274E-01	.273E-01	.272E-01	.270E-01	.269E-01	.264E-01	.254E-01	.248E-01	.242E-01
8.37	.269E-01	.268E-01	.267E-01	.265E-01	.264E-01	.263E-01	.258E-01	.248E-01	.242E-01	.237E-01
8.38	.263E-01	.261E-01	.260E-01	.259E-01	.258E-01	.257E-01	.252E-01	.242E-01	.236E-01	.231E-01
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8.41	.245E-01	.244E-01	.242E-01	.241E-01	.240E-01	.239E-01	.235E-01	.225E-01	.220E-01	.215E-01
8.42	.239E-01	.238E-01	.237E-01	.236E-01	.235E-01	.234E-01	.229E-01	.220E-01	.215E-01	.210E-01
8.43	.233E-01	.232E-01	.231E-01	.230E-01	.229E-01	.228E-01	.224E-01	.215E-01	.210E-01	.205E-01
8.44	.228E-01	.227E-01	.226E-01	.225E-01	.224E-01	.223E-01	.219E-01	.210E-01	.205E-01	.200E-01
8.45	.222E-01	.221E-01	.220E-01	.219E-01	.218E-01	.217E-01	.213E-01	.205E-01	.200E-01	.195E-01
8.46	.217E-01	.216E-01	.215E-01	.214E-01	.213E-01	.212E-01	.208E-01	.200E-01	.195E-01	.191E-01
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8.48	.207E-01	.206E-01	.205E-01	.204E-01	.203E-01	.202E-01	.199E-01	.190E-01	.186E-01	.182E-01
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8.50	.197E-01	.197E-01	.196E-01	.195E-01	.194E-01	.193E-01	.189E-01	.182E-01	.177E-01	.173E-01
8.51	.193E-01	.192E-01	.191E-01	.190E-01	.189E-01	.188E-01	.185E-01	.177E-01	.173E-01	.169E-01
8.52	.188E-01	.187E-01	.187E-01	.186E-01	.185E-01	.184E-01	.181E-01	.173E-01	.169E-01	.165E-01
8.53	.184E-01	.183E-01	.182E-01	.181E-01	.180E-01	.180E-01	.176E-01	.169E-01	.165E-01	.161E-01
8.54	.179E-01	.179E-01	.178E-01	.177E-01	.176E-01	.175E-01	.172E-01	.165E-01	.161E-01	.157E-01
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8.56	.171E-01	.170E-01	.170E-01	.169E-01	.168E-01	.167E-01	.164E-01	.157E-01	.153E-01	.150E-01
8.57	.167E-01	.166E-01	.165E-01	.165E-01	.164E-01	.163E-01	.160E-01	.153E-01	.150E-01	.146E-01
8.58	.163E-01	.162E-01	.162E-01	.161E-01	.160E-01	.159E-01	.156E-01	.150E-01	.146E-01	.143E-01
8.59	.159E-01	.158E-01	.158E-01	.157E-01	.156E-01	.156E-01	.153E-01	.146E-01	.143E-01	.139E-01
8.60	.155E-01	.155E-01	.154E-01	.153E-01	.153E-01	.152E-01	.149E-01	.143E-01	.139E-01	.136E-01
8.61	.152E-01	.151E-01	.151E-01	.150E-01	.149E-01	.148E-01	.145E-01	.139E-01	.136E-01	.133E-01
8.62	.148E-01	.147E-01	.147E-01	.146E-01	.145E-01	.145E-01	.142E-01	.136E-01	.133E-01	.130E-01
8.63	.145E-01	.144E-01	.143E-01	.143E-01	.142E-01	.141E-01	.139E-01	.133E-01	.129E-01	.126E-01
8.64	.141E-01	.140E-01	.140E-01	.139E-01	.138E-01	.138E-01	.135E-01	.129E-01	.126E-01	.123E-01
8.65	.138E-01	.137E-01	.136E-01	.136E-01	.135E-01	.135E-01	.132E-01	.126E-01	.123E-01	.120E-01
8.66	.134E-01	.134E-01	.133E-01	.133E-01	.132E-01	.131E-01	.129E-01	.123E-01	.120E-01	.117E-01
8.67	.131E-01	.131E-01	.130E-01	.129E-01	.129E-01	.128E-01	.126E-01	.120E-01	.117E-01	.115E-01
8.68	.128E-01	.127E-01	.127E-01	.126E-01	.126E-01	.125E-01	.123E-01	.117E-01	.114E-01	.112E-01
8.69	.125E-01	.124E-01	.124E-01	.123E-01	.123E-01	.122E-01	.120E-01	.115E-01	.112E-01	.109E-01
8.70	.122E-01	.121E-01	.121E-01	.120E-01	.120E-01	.119E-01	.117E-01	.112E-01	.109E-01	.106E-01
8.71	.119E-01	.118E-01	.118E-01	.117E-01	.117E-01	.116E-01	.114E-01	.109E-01	.106E-01	.104E-01
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8.73	.113E-01	.113E-01	.112E-01	.112E-01	.111E-01	.111E-01	.109E-01	.104E-01	.101E-01	.989E-02
8.74	.111E-01	.110E-01	.110E-01	.109E-01	.109E-01	.108E-01	.106E-01	.101E-01	.987E-02	.965E-02
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8.76	.105E-01	.105E-01	.104E-01	.104E-01	.103E-01	.103E-01	.101E-01	.964E-02	.940E-02	.918E-02
8.77	.103E-01	.102E-01	.102E-01	.101E-01	.101E-01	.100E-01	.984E-02	.940E-02	.917E-02	.895E-02
8.78	.100E-01	.998E-02	.994E-02	.989E-02	.984E-02	.979E-02	.960E-02	.917E-02	.894E-02	.873E-02
8.79	.979E-02	.974E-02	.969E-02	.965E-02	.960E-02	.955E-02	.936E-02	.895E-02	.872E-02	.852E-02
8.80	.955E-02	.950E-02	.946E-02	.941E-02	.937E-02	.932E-02	.914E-02	.873E-02	.851E-02	.831E-02
8.81	.932E-02	.927E-02	.923E-02	.918E-02	.914E-02	.909E-02	.891E-02	.851E-02	.830E-02	.810E-02
8.82	.909E-02	.905E-02	.901E-02	.896E-02	.891E-02	.887E-02	.869E-02	.830E-02	.809E-02	.790E-02
8.83	.887E-02	.882E-02	.878E-02	.874E-02	.869E-02	.865E-02	.848E-02	.810E-02	.789E-02	.770E-02
8.84	.865E-02	.861E-02	.857E-02	.852E-02	.848E-02	.844E-02	.827E-02	.790E-02	.769E-02	.751E-02
8.85	.844E-02	.840E-02	.836E-02	.831E-02	.827E-02	.823E-02	.807E-02	.770E-02	.750E-02	.732E-02
8.86	.823E-02	.819E-02	.815E-02	.811E-02	.807E-02	.803E-02	.787E-02	.751E-02	.732E-02	.714E-02
8.87	.803E-02	.799E-02	.795E-02	.791E-02	.787E-02	.783E-02	.767E-02	.732E-02	.713E-02	.696E-02
8.88	.783E-02	.779E-02	.775E-02	.772E-02	.768E-02	.764E-02	.748E-02	.714E-02	.695E-02	.679E-02
8.89	.764E-02	.760E-02	.756E-02	.752E-02	.749E-02	.745E-02	.730E-02	.696E-02	.678E-02	.662E-02
8.90	.745E-02	.741E-02	.738E-02	.734E-02	.730E-02	.726E-02	.712E-02	.679E-02	.661E-02	.645E-02
8.91	.727E-02	.723E-02	.719E-02	.716E-02	.712E-02	.708E-02	.694E-02	.662E-02	.644E-02	.629E-02
8.92	.709E-02	.705E-02	.701E-02	.698E-02	.694E-02	.691E-02	.677E-02	.645E-02	.628E-02	.613E-02
8.93	.691E-02	.687E-02	.684E-02	.681E-02	.677E-02	.674E-02	.660E-02	.629E-02	.612E-02	.597E-02
8.94	.674E-02	.670E-02	.667E-02	.664E-02	.660E-02	.657E-02	.643E-02	.613E-02	.597E-02	.582E-02
8.95	.657E-02	.654E-02	.650E-02	.647E-02	.644E-02	.640E-02	.627E-02	.598E-02	.582E-02	.567E-02
8.96	.641E-02	.637E-02	.634E-02	.631E-02	.628E-02	.624E-02	.611E-02	.583E-02	.567E-02	.553E-02
8.97	.625E-02	.621E-02	.618E-02	.615E-02	.612E-02	.609E-02	.596E-02	.568E-02	.553E-02	.539E-02
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8.01	.616E-01	.613E-01	.611E-01	.608E-01	.605E-01	.603E-01	.592E-01	.569E-01	.556E-01	.544E-01
8.02	.602E-01	.599E-01	.597E-01	.594E-01	.592E-01	.589E-01	.579E-01	.556E-01	.543E-01	.532E-01
8.03	.588E-01	.586E-01	.583E-01	.580E-01	.578E-01	.575E-01	.565E-01	.543E-01	.530E-01	.519E-01
8.04	.575E-01	.572E-01	.570E-01	.567E-01	.565E-01	.562E-01	.552E-01	.530E-01	.518E-01	.507E-01
8.05	.561E-01	.559E-01	.556E-01	.554E-01	.552E-01	.549E-01	.539E-01	.518E-01	.506E-01	.496E-01
8.06	.548E-01	.546E-01	.544E-01	.541E-01	.539E-01	.536E-01	.527E-01	.506E-01	.494E-01	.484E-01
8.07	.536E-01	.533E-01	.531E-01	.529E-01	.526E-01	.524E-01	.515E-01	.494E-01	.483E-01	.473E-01
8.08	.523E-01	.521E-01	.519E-01	.516E-01	.514E-01	.512E-01	.503E-01	.483E-01	.472E-01	.462E-01
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8.15	.444E-01	.442E-01	.440E-01	.438E-01	.437E-01	.435E-01	.427E-01	.410E-01	.400E-01	.392E-01
8.16	.434E-01	.432E-01	.430E-01	.428E-01	.426E-01	.425E-01	.417E-01	.400E-01	.391E-01	.383E-01
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8.20	.395E-01	.393E-01	.392E-01	.390E-01	.388E-01	.387E-01	.380E-01	.364E-01	.356E-01	.349E-01
8.21	.386E-01	.384E-01	.383E-01	.381E-01	.379E-01	.378E-01	.371E-01	.356E-01	.348E-01	.340E-01
8.22	.377E-01	.375E-01	.374E-01	.372E-01	.370E-01	.369E-01	.362E-01	.348E-01	.340E-01	.332E-01
8.23	.368E-01	.367E-01	.365E-01	.363E-01	.362E-01	.360E-01	.354E-01	.340E-01	.332E-01	.325E-01
8.24	.360E-01	.358E-01	.357E-01	.355E-01	.353E-01	.352E-01	.346E-01	.332E-01	.324E-01	.317E-01
8.25	.351E-01	.350E-01	.348E-01	.347E-01	.345E-01	.344E-01	.338E-01	.324E-01	.316E-01	.310E-01
8.26	.343E-01	.342E-01	.340E-01	.339E-01	.337E-01	.336E-01	.330E-01	.316E-01	.309E-01	.302E-01
8.27	.335E-01	.334E-01	.332E-01	.331E-01	.329E-01	.328E-01	.322E-01	.309E-01	.302E-01	.295E-01
8.28	.327E-01	.326E-01	.325E-01	.323E-01	.322E-01	.320E-01	.314E-01	.302E-01	.295E-01	.289E-01
8.29	.320E-01	.318E-01	.317E-01	.316E-01	.314E-01	.313E-01	.307E-01	.295E-01	.288E-01	.282E-01
8.30	.312E-01	.311E-01	.310E-01	.308E-01	.307E-01	.305E-01	.300E-01	.288E-01	.281E-01	.275E-01
8.31	.305E-01	.304E-01	.302E-01	.301E-01	.300E-01	.298E-01	.293E-01	.281E-01	.275E-01	.269E-01
8.32	.298E-01	.297E-01	.295E-01	.294E-01	.293E-01	.291E-01	.286E-01	.274E-01	.268E-01	.262E-01
8.33	.291E-01	.290E-01	.288E-01	.287E-01	.286E-01	.285E-01	.279E-01	.268E-01	.262E-01	.256E-01
8.34	.284E-01	.283E-01	.282E-01	.280E-01	.279E-01	.278E-01	.273E-01	.262E-01	.256E-01	.250E-01
8.35	.278E-01	.276E-01	.275E-01	.274E-01	.273E-01	.271E-01	.267E-01	.256E-01	.250E-01	.244E-01
8.36	.271E-01	.270E-01	.269E-01	.268E-01	.266E-01	.265E-01	.260E-01	.250E-01	.244E-01	.239E-01
8.37	.265E-01	.264E-01	.262E-01	.261E-01	.260E-01	.259E-01	.254E-01	.244E-01	.238E-01	.233E-01
8.38	.259E-01	.257E-01	.256E-01	.255E-01	.254E-01	.253E-01	.248E-01	.238E-01	.232E-01	.228E-01
8.39	.253E-01	.251E-01	.250E-01	.249E-01	.248E-01	.247E-01	.242E-01	.232E-01	.227E-01	.222E-01
8.40	.247E-01	.246E-01	.244E-01	.243E-01	.242E-01	.241E-01	.237E-01	.227E-01	.222E-01	.217E-01
8.41	.241E-01	.240E-01	.239E-01	.238E-01	.237E-01	.235E-01	.231E-01	.222E-01	.216E-01	.212E-01
8.42	.235E-01	.234E-01	.233E-01	.232E-01	.231E-01	.230E-01	.226E-01	.216E-01	.211E-01	.207E-01
8.43	.230E-01	.229E-01	.228E-01	.227E-01	.226E-01	.225E-01	.220E-01	.211E-01	.206E-01	.202E-01
8.44	.224E-01	.223E-01	.222E-01	.221E-01	.220E-01	.219E-01	.215E-01	.206E-01	.201E-01	.197E-01
8.45	.219E-01	.218E-01	.217E-01	.216E-01	.215E-01	.214E-01	.210E-01	.201E-01	.197E-01	.192E-01
8.46	.214E-01	.213E-01	.212E-01	.211E-01	.210E-01	.209E-01	.205E-01	.197E-01	.192E-01	.188E-01
8.47	.209E-01	.208E-01	.207E-01	.206E-01	.205E-01	.204E-01	.200E-01	.192E-01	.188E-01	.183E-01
8.48	.204E-01	.203E-01	.202E-01	.201E-01	.200E-01	.199E-01	.196E-01	.188E-01	.183E-01	.179E-01
8.49	.199E-01	.198E-01	.197E-01	.196E-01	.196E-01	.195E-01	.191E-01	.183E-01	.179E-01	.175E-01
8.50	.194E-01	.194E-01	.193E-01	.192E-01	.191E-01	.190E-01	.187E-01	.179E-01	.174E-01	.171E-01
8.51	.190E-01	.189E-01	.188E-01	.187E-01	.186E-01	.186E-01	.182E-01	.174E-01	.170E-01	.167E-01
8.52	.185E-01	.185E-01	.184E-01	.183E-01	.182E-01	.181E-01	.178E-01	.170E-01	.166E-01	.163E-01
8.53	.181E-01	.180E-01	.179E-01	.179E-01	.178E-01	.177E-01	.174E-01	.166E-01	.162E-01	.159E-01
8.54	.177E-01	.176E-01	.175E-01	.174E-01	.173E-01	.173E-01	.169E-01	.162E-01	.158E-01	.155E-01
8.55	.173E-01	.172E-01	.171E-01	.170E-01	.169E-01	.169E-01	.165E-01	.158E-01	.155E-01	.151E-01
8.56	.168E-01	.168E-01	.167E-01	.166E-01	.165E-01	.165E-01	.162E-01	.155E-01	.151E-01	.148E-01
8.57	.164E-01	.164E-01	.163E-01	.162E-01	.161E-01	.161E-01	.158E-01	.151E-01	.147E-01	.144E-01
8.58	.161E-01	.160E-01	.159E-01	.158E-01	.158E-01	.157E-01	.154E-01	.147E-01	.144E-01	.141E-01
8.59	.157E-01	.156E-01	.155E-01	.155E-01	.154E-01	.153E-01	.150E-01	.144E-01	.140E-01	.137E-01
8.60	.153E-01	.152E-01	.152E-01	.151E-01	.150E-01	.149E-01	.147E-01	.140E-01	.137E-01	.134E-01
8.61	.149E-01	.149E-01	.148E-01	.147E-01	.147E-01	.146E-01	.143E-01	.137E-01	.134E-01	.131E-01
8.62	.146E-01	.145E-01	.144E-01	.144E-01	.143E-01	.142E-01	.140E-01	.134E-01	.131E-01	.128E-01
8.63	.142E-01	.142E-01	.141E-01	.140E-01	.140E-01	.139E-01	.136E-01	.131E-01	.127E-01	.125E-01
8.64	.139E-01	.138E-01	.138E-01	.137E-01	.136E-01	.136E-01	.133E-01	.127E-01	.124E-01	.122E-01
8.65	.136E-01	.135E-01	.134E-01	.134E-01	.133E-01	.132E-01	.130E-01	.124E-01	.121E-01	.119E-01
8.66	.132E-01	.132E-01	.131E-01	.131E-01	.130E-01	.129E-01	.127E-01	.121E-01	.118E-01	.116E-01
8.67	.129E-01	.129E-01	.128E-01	.127E-01	.127E-01	.126E-01	.124E-01	.118E-01	.115E-01	.113E-01
8.68	.126E-01	.126E-01	.125E-01	.124E-01	.124E-01	.123E-01	.121E-01	.116E-01	.113E-01	.110E-01
8.69	.123E-01	.122E-01	.122E-01	.121E-01	.121E-01	.120E-01	.118E-01	.113E-01	.110E-01	.107E-01
8.70	.120E-01	.120E-01	.119E-01	.118E-01	.118E-01	.117E-01	.115E-01	.110E-01	.107E-01	.105E-01
8.71	.117E-01	.117E-01	.116E-01	.116E-01	.115E-01	.114E-01	.112E-01	.107E-01	.105E-01	.102E-01
8.72	.114E-01	.114E-01	.113E-01	.113E-01	.112E-01	.112E-01	.110E-01	.105E-01	.102E-01	.998E-02
8.73	.112E-01	.111E-01	.111E-01	.110E-01	.110E-01	.109E-01	.107E-01	.102E-01	.996E-02	.974E-02
8.74	.109E-01	.108E-01	.108E-01	.107E-01	.107E-01	.106E-01	.104E-01	.997E-02	.972E-02	.950E-02
8.75	.106E-01	.106E-01	.105E-01	.105E-01	.104E-01	.104E-01	.102E-01	.973E-02	.948E-02	.926E-02
8.76	.104E-01	.103E-01	.103E-01	.102E-01	.102E-01	.101E-01	.993E-02	.949E-02	.925E-02	.904E-02
8.77	.101E-01	.101E-01	.100E-01	.998E-02	.993E-02	.988E-02	.969E-02	.926E-02	.902E-02	.881E-02
8.78	.988E-02	.983E-02	.978E-02	.973E-02	.969E-02	.964E-02	.945E-02	.903E-02	.880E-02	.860E-02
8.79	.964E-02	.959E-02	.954E-02	.950E-02	.945E-02	.941E-02	.922E-02	.881E-02	.859E-02	.839E-02
8.80	.940E-02	.936E-02	.931E-02	.927E-02	.922E-02	.918E-02	.900E-02	.859E-02	.837E-02	.818E-02
8.81	.917E-02	.913E-02	.908E-02	.904E-02	.900E-02	.895E-02	.878E-02	.838E-02	.817E-02	.798E-02
8.82	.895E-02	.891E-02	.886E-02	.882E-02	.878E-02	.873E-02	.856E-02	.818E-02	.797E-02	.778E-02
8.83	.873E-02	.869E-02	.865E-02	.860E-02	.856E-02	.852E-02	.835E-02	.797E-02	.777E-02	.758E-02
8.84	.852E-02	.848E-02	.843E-02	.839E-02	.835E-02	.831E-02	.814E-02	.778E-02	.758E-02	.740E-02
8.85	.831E-02	.827E-02	.823E-02	.819E-02	.815E-02	.811E-02	.794E-02	.758E-02	.739E-02	.721E-02
8.86	.811E-02	.807E-02	.803E-02	.799E-02	.795E-02	.791E-02	.775E-02	.740E-02	.720E-02	.703E-02
8.87	.791E-02	.787E-02	.783E-02	.779E-02	.775E-02	.771E-02	.756E-02	.721E-02	.702E-02	.685E-02
8.88	.771E-02	.767E-02	.763E-02	.760E-02	.756E-02	.752E-02	.737E-02	.703E-02	.685E-02	.668E-02
8.89	.752E-02	.748E-02	.745E-02	.741E-02	.737E-02	.733E-02	.719E-02	.686E-02	.668E-02	.652E-02
8.90	.734E-02	.730E-02	.726E-02	.723E-02	.719E-02	.715E-02	.701E-02	.669E-02	.651E-02	.635E-02
8.91	.715E-02	.712E-02	.708E-02	.705E-02	.701E-02	.698E-02	.683E-02	.652E-02	.635E-02	.619E-02
8.92	.698E-02	.694E-02	.691E-02	.687E-02	.684E-02	.680E-02	.666E-02	.635E-02	.619E-02	.603E-02
8.93	.680E-02	.677E-02	.673E-02	.670E-02	.667E-02	.663E-02	.650E-02	.620E-02	.603E-02	.588E-02
8.94	.663E-02	.660E-02	.657E-02	.653E-02	.650E-02	.647E-02	.633E-02	.604E-02	.588E-02	.573E-02
8.95	.647E-02	.644E-02	.640E-02	.637E-02	.634E-02	.631E-02	.618E-02	.589E-02	.573E-02	.559E-02
8.96	.631E-02	.628E-02	.624E-02	.621E-02	.618E-02	.615E-02	.602E-02	.574E-02	.558E-02	.544E-02
8.97	.615E-02	.612E-02	.609E-02	.606E-02	.602E-02	.599E-02	.587E-02	.559E-02	.544E-02	.531E-02
8.98	.600E-02	.596E-02	.593E-02	.590E-02	.587E-02	.584E-02	.572E-02	.545E-02	.530E-02	.517E-02
8.99	.585E-02	.582E-02	.579E-02	.576E-02	.573E-02	.570E-02	.558E-02	.531E-02	.517E-02	.504E-02
9.00	.570E-02	.567E-02	.564E-02	.561E-02	.558E-02	.555E-02	.544E-02	.518E-02	.503E-02	.491E-02

TABLE FOR E= 0.31250E 01

Y=	.940E 00	.938E 00	.936E 00	.934E 00	.932E 00	.930E 00	.922E 00	.904E 00	.894E 00	.885E 00
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8.02	.593E-01	.590E-01	.587E-01	.585E-01	.582E-01	.580E-01	.570E-01	.547E-01	.535E-01	.523E-01
8.03	.579E-01	.576E-01	.574E-01	.571E-01	.569E-01	.566E-01	.556E-01	.534E-01	.522E-01	.511E-01
8.04	.566E-01	.563E-01	.561E-01	.558E-01	.556E-01	.553E-01	.544E-01	.522E-01	.510E-01	.500E-01
8.05	.553E-01	.550E-01	.548E-01	.545E-01	.543E-01	.541E-01	.531E-01	.510E-01	.498E-01	.488E-01
8.06	.540E-01	.537E-01	.535E-01	.533E-01	.530E-01	.528E-01	.519E-01	.498E-01	.487E-01	.477E-01
8.07	.527E-01	.525E-01	.523E-01	.520E-01	.518E-01	.516E-01	.507E-01	.487E-01	.476E-01	.466E-01
8.08	.515E-01	.513E-01	.511E-01	.508E-01	.506E-01	.504E-01	.495E-01	.475E-01	.465E-01	.455E-01
8.09	.503E-01	.501E-01	.499E-01	.497E-01	.494E-01	.492E-01	.484E-01	.464E-01	.454E-01	.444E-01
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8.13	.458E-01	.456E-01	.454E-01	.452E-01	.450E-01	.448E-01	.440E-01	.423E-01	.413E-01	.405E-01
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8.15	.437E-01	.435E-01	.434E-01	.432E-01	.430E-01	.428E-01	.420E-01	.403E-01	.394E-01	.386E-01
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8.18	.408E-01	.406E-01	.404E-01	.402E-01	.401E-01	.399E-01	.392E-01	.376E-01	.367E-01	.360E-01
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8.22	.371E-01	.370E-01	.368E-01	.366E-01	.365E-01	.363E-01	.357E-01	.342E-01	.334E-01	.327E-01
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8.25	.346E-01	.344E-01	.343E-01	.341E-01	.340E-01	.338E-01	.332E-01	.319E-01	.311E-01	.305E-01
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8.27	.330E-01	.329E-01	.327E-01	.326E-01	.324E-01	.323E-01	.317E-01	.304E-01	.297E-01	.291E-01
8.28	.322E-01	.321E-01	.319E-01	.318E-01	.317E-01	.315E-01	.310E-01	.297E-01	.290E-01	.284E-01
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8.30	.308E-01	.306E-01	.305E-01	.303E-01	.302E-01	.301E-01	.295E-01	.283E-01	.277E-01	.271E-01
8.31	.300E-01	.299E-01	.298E-01	.296E-01	.295E-01	.294E-01	.288E-01	.277E-01	.270E-01	.265E-01
8.32	.293E-01	.292E-01	.291E-01	.289E-01	.288E-01	.287E-01	.282E-01	.270E-01	.264E-01	.258E-01
8.33	.287E-01	.285E-01	.284E-01	.283E-01	.281E-01	.280E-01	.275E-01	.264E-01	.258E-01	.252E-01
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8.39	.249E-01	.248E-01	.246E-01	.245E-01	.244E-01	.243E-01	.239E-01	.229E-01	.223E-01	.219E-01
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8.41	.237E-01	.236E-01	.235E-01	.234E-01	.233E-01	.232E-01	.228E-01	.218E-01	.213E-01	.208E-01
8.42	.232E-01	.231E-01	.229E-01	.228E-01	.227E-01	.226E-01	.222E-01	.213E-01	.208E-01	.204E-01
8.43	.226E-01	.225E-01	.224E-01	.223E-01	.222E-01	.221E-01	.217E-01	.208E-01	.203E-01	.199E-01
8.44	.221E-01	.220E-01	.219E-01	.218E-01	.217E-01	.216E-01	.212E-01	.203E-01	.198E-01	.194E-01
8.45	.216E-01	.215E-01	.214E-01	.213E-01	.212E-01	.211E-01	.207E-01	.198E-01	.194E-01	.189E-01
8.46	.211E-01	.210E-01	.209E-01	.208E-01	.207E-01	.206E-01	.202E-01	.194E-01	.189E-01	.185E-01
8.47	.206E-01	.205E-01	.204E-01	.203E-01	.202E-01	.201E-01	.197E-01	.189E-01	.185E-01	.181E-01
8.48	.201E-01	.200E-01	.199E-01	.198E-01	.197E-01	.196E-01	.193E-01	.185E-01	.180E-01	.176E-01
8.49	.196E-01	.195E-01	.194E-01	.193E-01	.192E-01	.192E-01	.188E-01	.180E-01	.176E-01	.172E-01
8.50	.191E-01	.191E-01	.190E-01	.189E-01	.188E-01	.187E-01	.184E-01	.176E-01	.172E-01	.168E-01
8.51	.187E-01	.186E-01	.185E-01	.184E-01	.183E-01	.183E-01	.179E-01	.172E-01	.168E-01	.164E-01
8.52	.182E-01	.182E-01	.181E-01	.180E-01	.179E-01	.178E-01	.175E-01	.168E-01	.164E-01	.160E-01
8.53	.178E-01	.177E-01	.177E-01	.176E-01	.175E-01	.174E-01	.171E-01	.164E-01	.160E-01	.156E-01
8.54	.174E-01	.173E-01	.172E-01	.172E-01	.171E-01	.170E-01	.167E-01	.160E-01	.156E-01	.153E-01
8.55	.170E-01	.169E-01	.168E-01	.168E-01	.167E-01	.166E-01	.163E-01	.156E-01	.152E-01	.149E-01
8.56	.166E-01	.165E-01	.164E-01	.164E-01	.163E-01	.162E-01	.159E-01	.152E-01	.149E-01	.145E-01
8.57	.162E-01	.161E-01	.160E-01	.160E-01	.159E-01	.158E-01	.155E-01	.149E-01	.145E-01	.142E-01
8.58	.158E-01	.157E-01	.157E-01	.156E-01	.155E-01	.154E-01	.152E-01	.145E-01	.142E-01	.138E-01
8.59	.154E-01	.154E-01	.153E-01	.152E-01	.151E-01	.151E-01	.148E-01	.142E-01	.138E-01	.135E-01
8.60	.151E-01	.150E-01	.149E-01	.149E-01	.148E-01	.147E-01	.144E-01	.138E-01	.135E-01	.132E-01
8.61	.147E-01	.146E-01	.146E-01	.145E-01	.144E-01	.144E-01	.141E-01	.135E-01	.132E-01	.129E-01
8.62	.144E-01	.143E-01	.142E-01	.142E-01	.141E-01	.140E-01	.138E-01	.132E-01	.128E-01	.126E-01
8.63	.140E-01	.139E-01	.139E-01	.138E-01	.138E-01	.137E-01	.134E-01	.129E-01	.125E-01	.123E-01
8.64	.137E-01	.136E-01	.135E-01	.135E-01	.134E-01	.134E-01	.131E-01	.125E-01	.122E-01	.120E-01
8.65	.133E-01	.133E-01	.132E-01	.132E-01	.131E-01	.130E-01	.128E-01	.122E-01	.119E-01	.117E-01
8.66	.130E-01	.130E-01	.129E-01	.128E-01	.128E-01	.127E-01	.125E-01	.119E-01	.117E-01	.114E-01
8.67	.127E-01	.127E-01	.126E-01	.125E-01	.125E-01	.124E-01	.122E-01	.117E-01	.114E-01	.111E-01
8.68	.124E-01	.124E-01	.123E-01	.122E-01	.122E-01	.121E-01	.119E-01	.114E-01	.111E-01	.108E-01
8.69	.121E-01	.121E-01	.120E-01	.119E-01	.119E-01	.118E-01	.116E-01	.111E-01	.108E-01	.106E-01
8.70	.118E-01	.118E-01	.117E-01	.117E-01	.116E-01	.115E-01	.113E-01	.108E-01	.106E-01	.103E-01
8.71	.115E-01	.115E-01	.114E-01	.114E-01	.113E-01	.113E-01	.111E-01	.106E-01	.103E-01	.101E-01
8.72	.113E-01	.112E-01	.112E-01	.111E-01	.110E-01	.110E-01	.108E-01	.103E-01	.101E-01	.982E-02
8.73	.110E-01	.109E-01	.109E-01	.108E-01	.108E-01	.107E-01	.105E-01	.101E-01	.981E-02	.950E-02
8.74	.107E-01	.107E-01	.106E-01	.106E-01	.105E-01	.105E-01	.103E-01	.982E-02	.957E-02	.935E-02
8.75	.105E-01	.104E-01	.104E-01	.103E-01	.103E-01	.102E-01	.100E-01	.958E-02	.934E-02	.912E-02
8.76	.102E-01	.102E-01	.101E-01	.101E-01	.100E-01	.997E-02	.977E-02	.934E-02	.911E-02	.890E-02
8.77	.997E-02	.992E-02	.987E-02	.982E-02	.977E-02	.973E-02	.954E-02	.911E-02	.888E-02	.868E-02
8.78	.972E-02	.968E-02	.963E-02	.958E-02	.954E-02	.949E-02	.930E-02	.889E-02	.866E-02	.846E-02
8.79	.949E-02	.944E-02	.940E-02	.935E-02	.930E-02	.926E-02	.908E-02	.867E-02	.845E-02	.825E-02
8.80	.926E-02	.921E-02	.917E-02	.912E-02	.908E-02	.903E-02	.885E-02	.846E-02	.824E-02	.805E-02
8.81	.903E-02	.899E-02	.894E-02	.890E-02	.886E-02	.881E-02	.864E-02	.825E-02	.804E-02	.785E-02
8.82	.881E-02	.877E-02	.872E-02	.868E-02	.864E-02	.860E-02	.843E-02	.805E-02	.784E-02	.766E-02
8.83	.860E-02	.855E-02	.851E-02	.847E-02	.843E-02	.839E-02	.822E-02	.785E-02	.765E-02	.747E-02
8.84	.838E-02	.834E-02	.830E-02	.826E-02	.822E-02	.818E-02	.802E-02	.766E-02	.746E-02	.728E-02
8.85	.818E-02	.814E-02	.810E-02	.806E-02	.802E-02	.798E-02	.782E-02	.747E-02	.727E-02	.710E-02
8.86	.798E-02	.794E-02	.790E-02	.786E-02	.782E-02	.778E-02	.763E-02	.728E-02	.709E-02	.692E-02
8.87	.778E-02	.774E-02	.771E-02	.767E-02	.763E-02	.759E-02	.744E-02	.710E-02	.691E-02	.675E-02
8.88	.759E-02	.755E-02	.752E-02	.748E-02	.744E-02	.740E-02	.725E-02	.692E-02	.674E-02	.658E-02
8.89	.740E-02	.737E-02	.733E-02	.729E-02	.726E-02	.722E-02	.707E-02	.675E-02	.657E-02	.641E-02
8.90	.722E-02	.718E-02	.715E-02	.711E-02	.708E-02	.704E-02	.690E-02	.658E-02	.641E-02	.625E-02
8.91	.704E-02	.701E-02	.697E-02	.694E-02	.690E-02	.687E-02	.673E-02	.642E-02	.625E-02	.609E-02
8.92	.687E-02	.683E-02	.680E-02	.676E-02	.673E-02	.670E-02	.656E-02	.626E-02	.609E-02	.594E-02
8.93	.670E-02	.666E-02	.663E-02	.660E-02	.656E-02	.653E-02	.640E-02	.610E-02	.594E-02	.579E-02
8.94	.653E-02	.650E-02	.646E-02	.643E-02	.640E-02	.637E-02	.624E-02	.594E-02	.579E-02	.564E-02
8.95	.637E-02	.634E-02	.630E-02	.627E-02	.624E-02	.621E-02	.608E-02	.579E-02	.564E-02	.550E-02
8.96	.621E-02	.618E-02	.615E-02	.611E-02	.608E-02	.605E-02	.593E-02	.565E-02	.550E-02	.536E-02
8.97	.605E-02	.602E-02	.599E-02	.596E-02	.593E-02	.590E-02	.578E-02	.550E-02	.536E-02	.522E-02
8.98	.590E-02	.587E-02	.584E-02	.581E-02	.578E-02	.575E-02	.563E-02	.537E-02	.522E-02	.509E-02
8.99	.575E-02	.572E-02	.569E-02	.567E-02	.564E-02	.561E-02	.549E-02	.523E-02	.509E-02	.496E-02
9.00	.561E-02	.558E-02	.555E-02	.552E-02	.549E-02	.546E-02	.535E-02	.510E-02	.496E-02	.483E-02

0.00549.

0.00483

APPENDIX 11

Tables 11.1, 11.2 and 11.3 (Golterman, 1969) used in the calculation of total carbon dioxide (T.C.) and total alkalinity (T.A.) of fresh water.

Table II.1

Factors (β) used for calculating carbonate alkalinity (C.A.) from total alkalinity (T.A.), according to C.A. = T.A. - 0.01 β at different values of pH and conductivity of the original water sample.

pH	Conductivity (μmho/cm) 0-40	40-300	300-350
8.8	0	0	0
8.9	1	1	1
9.0	1	1	1
9.1	1	1	1
9.2	1	1	1
9.3	1	1	1
9.4	2	2	2
9.5	2	2	2
9.6	3	3	3
9.7	4	4	4
9.8	4	5	5
9.9	6	6	6
10.0	7	7	7
10.1	9	9	9
10.2	11	11	12
10.3	14	14	15
10.4	17	18	19

Table II.2

Factors σ for calculating total carbon dioxide (T.C.) from carbonate alkalinity (C.A.), according to T.C. = T.A. x σ_1 for different values of pH and conductivity of the original water sample.

pH	Conductivity (μmho/cm)						
	0-10	10-40	40-110	110-200	200-315	315-430	430-550
6.4	1.96	1.93	1.93	1.90	1.89	1.88	1.86
6.5	1.76	1.74	1.74	1.72	1.71	1.70	1.69
6.6	1.60	1.59	1.59	1.57	1.56	1.55	1.55
6.7	1.48	1.46	1.46	1.45	1.45	1.44	1.43
6.8	1.38	1.37	1.37	1.36	1.35	1.35	1.34
6.9	1.30	1.29	1.29	1.29	1.28	1.28	1.27
7.0	1.24	1.23	1.23	1.23	1.22	1.22	1.22
7.1	1.19	1.19	1.18	1.18	1.18	1.17	1.17
7.2	1.15	1.15	1.15	1.14	1.14	1.14	1.14
7.3	1.12	1.12	1.12	1.11	1.11	1.11	1.11
7.4	1.09	1.09	1.09	1.09	1.09	1.09	1.09
7.5	1.07	1.07	1.07	1.07	1.07	1.07	1.07
7.6	1.06	1.06	1.06	1.06	1.05	1.05	1.05
7.7	1.05	1.05	1.04	1.04	1.04	1.04	1.04
7.8	1.04	1.04	1.03	1.03	1.03	1.03	1.03
7.9	1.03	1.03	1.03	1.03	1.02	1.02	1.02
8.0	1.02	1.02	1.02	1.02	1.02	1.02	1.02
8.1	1.01	1.01	1.01	1.02	1.01	1.01	1.01
8.2	1.01	1.01	1.01	1.01	1.01	1.01	1.01
8.3	1.00	1.00	1.00	1.00	1.00	1.00	1.00
8.4	1.00	1.00	1.00	1.00	1.00	1.00	1.00
8.5	1.00	.99	.99	.99	.99	.99	.99
8.6	.99	.99	.99	.99	.99	.99	.98
8.7	.99	.98	.98	.98	.98	.98	.98
8.8	.98	.98	.98	.97	.97	.97	.97
8.9	.97	.97	.97	.97	.97	.96	.96
9.0	.96	.96	.96	.96	.96	.95	.95
9.1	.95	.95	.95	.95	.94	.94	.94
9.2	.94	.94	.94	.93	.93	.93	.93
9.3	.93	.93	.92	.92	.92	.91	.91
9.4	.91	.91	.91	.90	.90	.90	.89
9.5	.90	.89	.89	.88	.88	.87	.87
9.6	.88	.87	.87	.86	.85	.85	.85
9.7	.85	.85	.84	.84	.83	.83	.82
9.8	.83	.82	.82	.81	.80	.80	.79
9.9	.80	.79	.79	.78	.77	.77	.76
10.0	.77	.76	.76	.75	.75	.74	.74
10.1	.74	.74	.73	.72	.72	.71	.71
10.2	.72	.71	.70	.70	.69	.69	.68
10.3	.69	.68	.68	.67	.66	.66	.65
10.4	.66	.65	.65	.64	.64	.63	.63

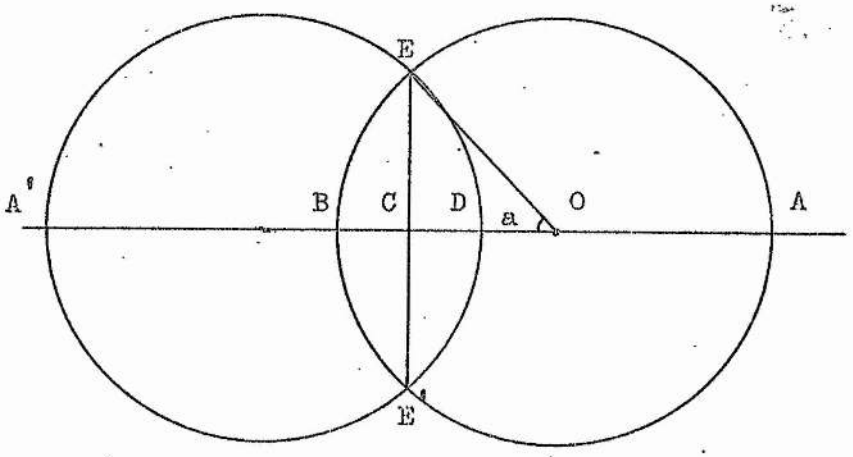
Table II.3

End point pH values at 20°C for the total alkalinity titration

Total CO_2 mML^{-1}	End point pH
0.05	5.36
0.08	5.25
0.10	5.20
0.15	5.11
0.2	5.05
0.3	4.96
0.4	4.90
0.5	4.85
0.6	4.81
0.8	4.74
1.0	4.69
1.2	4.65
1.5	4.61
2.0	4.54
2.5	4.49
3.0	4.46
4.0	4.39
5.0	4.34
6.0	4.30
7.0	4.27
8.0	4.24
10.0	4.19

APPENDIX III

The Calculation of the Volume of the Laminar Boundary layer Over a Leaf Disc



Consider a disc A'EDE' of radius r . Then circle AEBE' is a circle of radius r, with its centre O on the axis A'BCDOA where L = the distance on this axis from the leading edge A'.

$$AB = 2r \ , \quad AO = r \ , \quad A'B = AD = L$$

$$OD = AD - OA = L - r$$

$$BD = AB - AD = 2r - L$$

$$\Rightarrow \quad BC = CD = \frac{BD}{2} = r - \frac{L}{2}$$

$$\Rightarrow \quad OC = OD + DC = (L - r) + (r - \frac{L}{2}) = \frac{L}{2}$$

$$\Rightarrow \quad AC = AD + DC = L + (r - \frac{L}{2}) = r + \frac{L}{2}$$

In circle AEBE',

$$EC^2 = BC \times CA = (r - \frac{L}{2})(r + \frac{L}{2}) = r^2 - \frac{L^2}{4}$$

$$\Rightarrow \quad EC = (r^2 - \frac{L^2}{4})^{\frac{1}{2}}$$

In DECD,

$$\frac{EC}{CO} = \tan a = \frac{2(r^2 - \frac{L^2}{4})^{\frac{1}{2}}}{L}$$

$$\Rightarrow \quad a = \tan^{-1} \frac{2(r^2 - \frac{L^2}{4})^{\frac{1}{2}}}{L}$$

The angle subtended by EE' from O , equals 2a

$$\Rightarrow \text{length of EE' arc} = \frac{2a \times 2\pi r}{2\pi} = 2ar$$

Height of section EBE' = d, the depth of the laminar boundary layer given by $d = k L^{\frac{1}{2}}$, where $k = 5 U^{-\frac{1}{2}} \nu^{\frac{1}{2}}$

Area of section EBE' = $2 k r L^{\frac{1}{2}} a$.

Volume of boundary layer over disc A'EDE' will be given by;

$$v = \int_0^{2r} 2 k r L^{\frac{1}{2}} a \, . \, dL$$

Which equals;

$$v = 10 V^{\frac{1}{2}} U^{-\frac{1}{2}} \int_0^{2r} L^{\frac{1}{2}} \tan^{-1} \left[2L^{-1} (r^2 - \frac{L^2}{4})^{\frac{1}{2}} \right] dL$$